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TREATMENT OF CANCER AND MORTALITY RATES.

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DESPONDENCY has been expressed in several quarters that improved treatment and apparently successful efforts to persuade those afflicted with cancer to present themselves early for diagnosis and treatment have not been reflected in a fall in mortality rates. Much of this disappointment seems due to the feeling that the only successful outcome of treatment is that which results in an absolute cure and other possibilities appear largely to have been neglected. Although the complete extirpation of the cancer focus so that the patient is subsequently subject to normal mortality risk only is the ideal to be aimed at, yet treatment short of this may still confer such benefit that it may justly be described as successful. Even though the word cure be inadmissible the disease may be arrested for a longer or a shorter time and the expectation of life considerably increased; at the worst treatment may make existence more comfortable without affecting the ultimate prognosis. Mortality rates can only be affected by results under the first two of these heads, and though it is obvious that the result of cure should be reflected in an early fall in mortality, the effect of prolongation of life is more complicated. Without attempting to differentiate between the effects of a complete cure or a prolongation of life this note will be concerned with the effect upon mortality rates of a hypothetical increase in the mean duration of survival of some typical cancers. It will be assumed that the average expectation of life of all cancer cases has been increased by two and a half years and the effect of this upon the mortality rates at the following sites will be considered; the results differ for cancer of different sites and depend upon variations in the curve of mortality with age. Five sites, each showing a different type of age mortality curve, have been selected (Fig. 1). First, cancer of the stomach in males, for which the cancer mortality curve follows the classical type for non-genital cancers in that it starts at a low rate in early adult life and steadily and rapidly increases with increasing age. Second, cancer of the lung in males, for which the curve follows the classical pattern until about the age of 55 when the upward slope decreases until there is a final fall. Third, cancer of the prostate, for which mortality at early ages is small, but after 50 the death-rate increases with extreme rapidity until old age. Fourth, cancer of the uterus, for which the rate increases rapidly in early adult life, less during middle age and changes little after 60 years of age. Finally cancer of the female breast, where the rate increases with each increase in age but more rapidly below the age of 45. The mortality records of 1952 have been used in five-year age-groups from 30–74 and for all ages over 75. To estimate the effect of an all-

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round increase in expectation of life of two and a half years, one half of the deaths recorded in each age-group for each type of cancer have been transferred to the age-group above and from these two sets of figures mortality rates per million living have been calculated, the rates resulting from the hypothetical extension

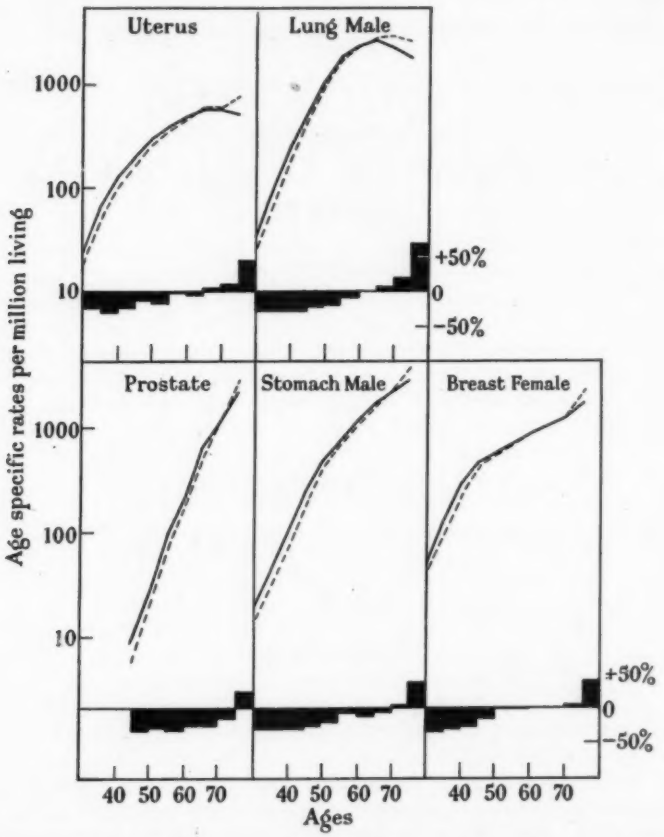

FIG. 1.—Age specific mortality rates per million living shown on a logarithmic curve for cancer of: Males—lung, prostate and stomach; Females—breast and uterus.
——— Recorded rates in England and Wales 1952.
– – – – – Calculated rates, assuming 2·5 years extension of life in each individual case; the histogram at the foot of each curve shows the percentage change in the rate at each age group.

of life being expressed as a percentage of the observed rates (Table I). It is seen that at the earliest ages the increased expectation of life lowers the mortality rates, while above 75 years the hypothetical rate considerably exceeds the observed; between these extremes the relation of the two rates varies with the type of age mortality rate curve. The effect of this change in expectation of life at different ages is shown graphically for each site in the diagram.

TABLE I.

Age.	Stomach, males (151).			Lung, males (162, 163).			Prostate (177).			Uterus (171, 172, 174).			Breast, females (170).		
	A.	B.	C.	A.	B.	C.	A.	B.	C.	A.	B.	C.	A.	B.	C.
30–	20	14	70	35	25	70				23	17	74	47	29	62
35–	47	33	70	96	64	69				64	44	69	129	88	68
40–	109	77	70	255	173	68				123	93	76	299	212	71
45–	263	189	72	555	411	74	9	6	67	179	154	86	463	386	83
50–	509	408	80	1170	909	77	28	20	71	310	253	82	569	536	94
55–	766	695	91	1942	1688	87	99	66	67	387	367	95	719	677	94
60–	1205	1049	87	2334	2299	98	231	174	75	503	467	93	874	836	96
65–	1792	1631	91	2700	2773	103	637	460	72	544	564	104	1029	1022	99
70–	2314	2348	101	2288	2939	128	1207	1027	85	557	612	110	1223	1243	102
75+	3031	4129	136	1725	2662	154	2271	2845	125	531	769	145	1678	2190	135

Column A : Age specific death rate per million living in 1952.
" B : " " " " " " " (assuming an increase in life of 2½ years for each person).*
" C : Proportion of B to A as a percentage.

* If the increase is made proportional to the expectation of life at the appropriate age group but the average for all person remains the same, the result is only slightly different. Compared with the previously calculated rate the new one is still lower at earlier ages but approaches and exceeds the observed earlier, while the final increase is less.

Considering first the more general case of cancer of the stomach in males we see that between 30 and 50 years of age the hypothetical death rate is about 30 per cent below the observed, between 55 years and 70 years the difference is reduced to about 10 per cent, while for the age-group 70–74 the rates are practically the same and for the last group, those over 75, the hypothetical rate is naturally very much higher and is 36 per cent above the observed mortality. In cancer of the prostate, the new rate varies within 70 and 80 per cent of the observed until the age of 65 years, when it rapidly rises to 125 per cent over 75 years. In contradistinction to this are cancer of the lung in males and cancer of the uterus, for which sites the curves show mortality to increase until the seventh decade of life and thereafter to fall or remain stationary. In both cancers the difference between the recorded mortality rates and the rates calculated from our assumptions progressively diminishes between 30 and 60 years of age. The assumed extension of life actually increases the mortality rates in the three age-groups over 65 years, more strikingly in the case of the lung, where the real rates over 65 fall, than in the case of the uterus, where they remain steady. The mortality curve of cancer of the female breast rises steeply between 30 and 39 years of age and continues from 45 to 69 years at a much reduced gradient, when it again ascends. Between 30–34 years of age the supposed increase of life reduces the mortality rate by nearly 40 per cent, but before 50 is reached the difference has fallen to 6 per cent and thereafter until 75 the rates are scarcely affected.

The addition of two and a half years of life to every person who dies from cancer would prove no mean medical feat and would be accompanied by a considerable change for the better in the crude survival rate. In Table II are shown first the five-year crude survival rates for the sites discussed above, for all cases, treated and untreated, which were registered under the Cancer Registration Scheme in 1945 and 1946 ; and second, the calculated rate assuming an extension of life of two and a half years for each cancer patient. (The corrected rates, according to the method of Berkson, show similar relationships.)

TABLE II.

	1945–6 Cancer Registrations 5-year survival rate. %	Calculated rate assuming 2·5 years extension of life. %	Increase. %
Stomach, male	4·6	6·8	48
Lung, male	1·5	3·0	100
Prostate	15·0	31·0	48
Uterus (cervix only)	32·0	44·0	37
Breast, female	31·0	49·0	58

Recent mortality records show that at several sites the distribution of cancer deaths among the different age groups is changing, often without commensurate alterations in the overall mortality rate. At only a few sites has the mortality rate over 75 years declined, and these sites contribute little to the total cancer picture ; lips, liver and skin in both sexes, and tongue in males, are the most noteworthy ; at nearly all other sites the mortality rate at this older age has either risen or remained steady during the last twenty years. At the following important sites the rates below 75 years are falling, while those above have risen or show no change. Males : oesophagus, intestine, rectum, mouth, larynx and

prostate. Females: mouth, larynx, stomach, intestine, rectum and uterus. Both sexes: "all other sites," excluding cancer of the lung. Cancer of the male larynx, the age specific rates of which are shown in Fig. 2, illustrates well this pattern. Between 1936 and 1952 the rates between 45 and 74 years of age have fallen by approximately 30 per cent. Some beneficial change must be taking place, though its nature is obscure and even its existence not apparent unless a

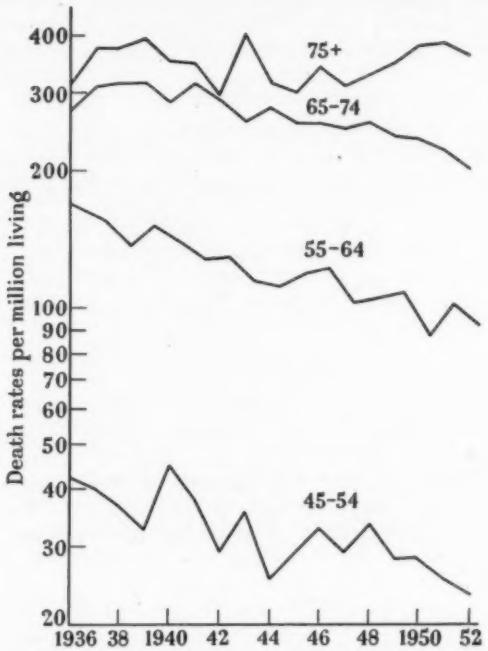

FIG. 2.—Cancer of male larynx, England and Wales, 1936–1952. Mortality rates per million living shown on a logarithmic scale for 5-year age-groups.

detailed analysis of the records is undertaken. The effect could be produced by any one of the following mechanisms or by a combination of all three:

(*a*) A shift in the mean age of incidence to older groups, caused perhaps by a waning effect of the hypothetical cancer-producing factor, or by a change in habits at younger ages which postpones full contact with the factor until later life.

(*b*) An increased expectation of life for all cancer patients as outlined above, due either to improved or earlier treatment, to increased individual resistance, or a decline in the "malignancy" of the cancer.

(*c*) A greater proportion of complete cures affecting mainly the younger ages and removing them from the cancer mortality tables.

To estimate the relative importance of any single mechanism it would be necessary to integrate incidence and survival rates with mortality experience, and this will not be possible until cancer registration covers a broader field than

at present, when we should be in a position to realise not only that something is happening but also determine what is happening.

This note is restricted solely to the effects of an extension of life upon mortality rates, the complete analysis should take account of cures and consequent reduction in numbers of persons dying from cancer as well as possible changes in total or average age incidence. Sufficient has, however, been said to show that any deduction drawn from changes in mortality rates must be related to the trends of the age mortality rate curve and that the absence of any general fall in overall rates is not incompatible with considerable improvements in prognosis.

SUMMARY.

The effect on cancer mortality rates of an increase in the duration of survival from cancer at certain typical sites is discussed. The resulting changes in the rates are not constant and vary with the form of the mortality curve.

CANCER VENTRICULI MORTALITY IN DIFFERENT POPULATION GROUPS. CANCER VENTRICULI MORTALITY IN VARIOUS PARTS OF OSLO, 1930–50.

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IT is generally recognized that total mortality varies considerably for different population groups. It has, however, become more evenly distributed in recent years, but is still higher in the cities than in the country. The present paper is an attempt to examine statistically the mortality among various population groups for a particular cause of death—cancer ventriculi.

The suggestion has frequently been made that environment may be one of the factors governing cancer mortality. It was, for example, mentioned in the rather extensive investigation made by Wåler in 1931. He concludes that an hereditary disposition may be one of the contributory causes of cancer. He presents some evidence in support of this conclusion, but later points out that the majority of factors which indicate an hereditary disposition might just as well be interpreted in favour of the hypothesis that cancer is environmentally determined.

Wåler (1931) states that it may be assumed that the localization of cancer is hereditary, and he mentions cancer of the breast and uterus as examples, but, on the whole, he discusses cancer mortality without regard for the possibility that the situation might differ from one localization to another. Engelstad writes : "It is not correct, in the light of present knowledge, to speak of 'cancer families' as has been done previously. However, there may be some justification for the expressions 'breast cancer families' or 'stomach cancer families,' etc."

Before discussing our material from different parts of Oslo to examine the possibility whether cancer ventriculi mortality in particular is environmentally determined, we shall consider some similar investigations which have been made by others.

The problem itself is not new. The first Norwegian social statistician, Eilert Sundt, demonstrated in the middle of the last century that mortality varied from one part of the country to another. But in recent years such investigations have tended to become more concentrated on particular causes of death. Since the campaign against tuberculosis has made this a disease of only secondary significance, cancer has come more to the fore in this connection.

Among the older Norwegian publications there is an extensive article by Thoner (1924). This investigation covers 120 cases and it is interesting because Thoner, in a carefully examined material, tries to answer the question as to the relation between cancer and living conditions, hygiene, age geography, geology and heredity. His conclusion is that there seem to be some cancer localities, especially in Hemsedal, and that this would seem to indicate the influence of environment.

In England, Stocks (1950) has found that cancer ventriculi mortality is particularly high in the northern part of the country. If the mortality for the entire country is put at 100, the figures for individual parts vary from 55 to 130. Stocks found further that mortality from cancer ventriculi was higher in the groups with a low standard of living than in those with a higher standard.

Stockholm's Municipal Bureau of Statistics has recently (1953) published figures on total cancer mortality and mortality from individual forms of cancer for the years 1940/41 and 1945/46, with separate figures for (1) Stockholm, (2) Gothenburg and Malmø, (3) other cities and (4) country districts. Corresponding figures have been calculated for Denmark (1949/50) and Norway (1946/47).

The results show that among the age-groups of interest in this respect, those over 55 years, the total cancer mortality in all three countries is decidedly higher in the capitals than in the medium-sized cities, and higher in the medium cities than in the small ones, and again higher in the small cities than in the country districts. The difference is most pronounced for men.

This tendency toward increasing cancer mortality with increasing agglomeration is not really surprising. In these age-groups the total mortality is also higher in the cities than in the country and is particularly high in the largest cities. The only exception to this rule is for women in Sweden. The causes of the higher mortality in the cities as compared to the country have not been studied to any extent, but there is little reason to expect cancer mortality to differ from other causes of death.

However, if we consider cancer ventriculi as a separate group, we find a different relationship between the country districts and the three groups of cities. For men there is no decided difference between mortality from cancer ventriculi in Stockholm, Gothenburg and Malmø, other cities and the country districts. But the figures for 1945/46 show that mortality for men over 60 was lower in Stockholm than in the other places. For women this relationship is striking, mortality for the age-groups 55–70 in both periods of the investigation is decidedly highest in the country and lowest in Stockholm.

In evaluating the figures from Sweden it is important to take into consideration the fact that, in the Swedish mortality statistics for 1946/47, deaths from cancer of the liver are grouped together with deaths from cancer of the stomach.

In Norway, according to these Swedish calculations, mortality from cancer ventriculi in 1946/47 was decidedly lowest in Oslo with a particularly marked difference for women, and the tendency in Denmark in 1949/50 was in the same direction and was very decided for both sexes.

In the period 1930–50 it is possible to compare cancer ventriculi mortality in the country and in the cities for the census years 1930, 1946 and 1950. As the census counts are made in December in each of these years it is reasonable to regard the figures as an expression of the mean population for 1930/31, 1946/47 (the same figures used by Olinder (1953) in the Swedish investigation) and 1950/51. We have calculated the figures for cancer ventriculi mortality for these periods with the result shown in Table I.

The figures for 1946/47 agree completely with those of Olinder (1953). They indicate a tendency toward lower cancer ventriculi mortality in the cities than in the country. For women between the ages of 40 and 70 there is even a decided difference between the mortality in the cities and in the country. The excess mortality for women of this age is just as pronounced in 1930/31. The same age

TABLE I.—*Number of Deaths from Cancer Ventriculi per year per* 100,000 *of the Native Population in Norway.*

Age in years.	1930/31.				1946/47.				1950/51.			
	Country.		Cities.		Country.		Cities.		Country.		Cities.	
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
Under 30	0·1	0·6	0·3	—	0·3	0·2	—	—	—	0·2	0·2	—
30–39	8	7	10	3	6	3	6	7	5	4	4	4
40–49	43	33	36	21	20	18	25	16	21	16	23	14
50–59	126	86	137	51	78	49	77	45	75	40	75	37
60–69	328	224	342	179	233	138	228	99	186	119	185	115
70 and over	559	425	595	488	551	383	542	497	555	404	537	296
Total	64	53	60	47	57	44	56	42	56	44	55	37

groups in 1950/51 show little difference between cities and country, but in contrast to 1930/31 and 1946/47 there is an excess mortality for the country population in the age-group 70 and over.

As regards cancer ventriculi mortality for men, there is no tendency toward a systematic difference between cities and country. What can be concluded is that the mortality is not significantly higher in the cities than in the country for any of the two-year periods as it is for the other forms of cancer.*

There does not seem to be any systematic change in the relationship between cancer ventriculi mortality in the cities and in the country in the course of the period 1930/31–1950/51. According to the medical statistics there has been a very considerable reduction in cancer ventriculi mortality during the 20-year period in both the cities and the country. This is illustrated in Table II.

TABLE II.—*Percentage Reduction in Cancer Ventriculi Mortality from* 1930/31–1950/51.

Age.	Country.		Cities.	
	M.	F.	M.	F.
50–59	40·5	57·5	45·3	27·5
60–69	43·3	46·9	45·9	35·8
70 and over	0·7	4·9	9·7	39·3

It seems that cancer ventriculi mortality in the age-groups between 50 and 70 was about 40 per cent lower in 1950/51 than in 1930/31, while the reduction is more uneven for the ages over 70. For most of the groups the reduction

* The death rates per 100,000 population in 1950–51 for cancer of the stomach in England and Wales (calculated from data given in the Registrar-General's Statistical Reviews for those years) were :

	Males.				Females.			
	25–	45–	65–	75+	25–	45–	65–	75+
Conurbations	6	63	213	293	4	31	127	244
Smaller urban areas	6	65	216	304	3	29	125	240
Rural districts	4	53	191	287	3	27	113	230

has taken place equally rapidly from 1946/47 to 1950/51 as from 1930/31 to 1946/47, so there is every reason to believe that cancer ventriculi mortality will decrease still more.

In other words the statistics indicate clearly that the city environment affects cancer ventriculi in a different manner from other types of cancer. According to the statistics this is apparently true; but statistics are never better than the material on which they are based; and diagnosis may be a weak point in the mortality statistics. In the above mentioned publication from Stockholm's Bureau of Statistics it is stated that the distribution of deaths among causes of death is not exact for the country districts. There is reason to believe that the diagnoses are not quite exact in the cities either, but in Denmark and Norway also they are probably more correct in the cities than in the country.

We shall return later to the possibility that incorrect diagnosis may be a significant cause of the statistically lower mortality from cancer ventriculi in the cities. For the present we use the Swedish calculations as statistical indications that this is a real situation, or, as Olinder states: "It is obvious that the strength and nature of the factors which further or inhibit the disease are essentially different in the cities and in the country districts." But he adds: "Obviously, a more accurate grouping of the population is necessary in order to determine a causal connection in the question of the origin of the disease."

Our problem is really a particularization of the Swedish calculations and is based on Stocks' (1950) results from England: Can it be demonstrated that mortality from cancer ventriculi differs among various population groups in Oslo?

It appears that cancer ventriculi is a special case as regards the relationship between mortality in the cities and in the country. It is highest in the country, while the reverse is true of most other causes of death. On the other hand, Stocks' (1950) figures indicated that cancer ventriculi, like most other causes of death, takes its heaviest toll of the poorest level of the population. The apparent conflict between these two statements makes it difficult to determine what factors are at work—and what, for example, is the situation for different parts of Oslo.

Primarily with the idea of examining this latter question we have made a special compilation of all deaths from cancer ventriculi in Oslo (without Aker) from 1930–50 from the records of the Oslo Bureau of Public Health and from death certificates. We shall first examine the figures for the individual age groups for the population of Oslo as a whole (Table III).

TABLE III.—*Deaths from Cancer Ventriculi in Oslo (without Aker)* 1930–50.

Age.	Men. Number of deaths.	Men. Native population December 1, 1937.	Men. Deaths per 100,000 population per year 1930–50.	Women. Number of deaths.	Women. Native population December 1, 1937.	Women. Deaths per 100,000 population per year 1930–50.
Under 20	2	—	—	0	—	—
20–29	2	26,319	0·4	3	31,439	0·5
30–39	20	24,754	4	25	30,469	4
40–49	117	19,383	29	82	23,234	17
50–59	255	12,094	100	165	16,705	47
60–69	452	8,391	257	369	12,425	141
70–79	384	3,801	481	454	6,951	311
80 and over	135	870	739	279	2,028	655

During the course of the 21-year period, 1930–50, mortality from cancer ventriculi has decreased. It is, therefore, not surprising that cancer ventriculi mortality for the period as a whole is somewhat higher than the corresponding figures in the Swedish calculations for 1946/47. However, the relationship between the age-groups and the proportional mortality for men and women remains about the same.

We have calculated cancer ventriculi mortality for the east and west sides of the city separately. The division has been made by parishes as follows :

West side parishes :		East side parishes :	
Vår Frelser	Fagerborg	Jakob	Grønland
Trefoldighet	Gamle Aker	Sagene	Kampen
Frogner	Markus	Torshov	Gamlebyen
Uranienborg		Paulus	Vålerenga
		Petrus	

Table IV shows mortality for the populations of the two parts of the city.

TABLE IV.—*Cancer Ventriculi Mortality on the East and West Sides of Oslo,* 1930–50.

Deaths per year per 100,000 population (12/1–1937).

	West side.			East side.			Entire city.		
Age.	M.	F.	Total.	M.	F.	Total.	M.	F.	Total.
50–59	70	39	51	127	56	88	100	47	69
60–69	198	103	139	305	186	239	257	141	188
70–79	456	261	324	503	371	422	481	311	371

For the present we shall consider only the typical cancer ventriculi age groups. The age groups 50–79 years included more than $\frac{3}{4}$ of all deaths from cancer ventriculi in Oslo during the period 1930–50.

The figures reveal at once that, if the material, *i.e.* the diagnosis, is correct, cancer ventriculi mortality between the ages of 50 and 80 is decidedly higher on the east side of the city than on the west. For each of the three age-groups and for both sexes it is obvious that the mortality figures for the west side are lower and those for the east side higher than the corresponding figures for the city as a whole. The difference in cancer ventriculi mortality between the west and east sides is more than 50 per cent for the two youngest age-groups, for the group 70–79 years the difference is not quite as marked.

If we study the figures more closely we find that the difference between cancer ventriculi mortality on the west and east sides of the city is somewhat greater for men than for women in the age-group 50–59 years, but considerably greater for women than for men in the age-groups 60–69 and 70–79 years.

There are no census figures for each separate year as regards age distribution in the various parishes. For this reason we have used the municipal census figures of December 1, 1937 as a basis for calculating mortality. It is the census which is closest to the middle of the period 1930–50. In order to determine whether chance variations in the sex distribution play an important part, we have

also calculated the mortality on the basis of an average of the age distributions on December 1, 1930, December 1, 1937, and October 1, 1948. This gives the figures in Table V.

TABLE V.—*Mortality from Cancer Ventriculi on the West and East Sides of Oslo*, 1930–50.

Deaths per year per 100,000 population 12/1 1930–12/1 1937–10/1 1948.

	West side.			East side.			Entire city.		
Age.	M.	F.	Total.	M.	F.	Total.	M.	F.	Total.
50–59	64	37	48	108	49	76	88	43	62
60–69	201	102	138	299	178	230	253	137	184
70–79	431	243	302	456	350	392	445	291	346

It is found that the mortality figures on the average are slightly lower when based on this average age distribution than when the age distribution on December 1, 1937, is used as a basis. However, the reduction affects the east and west side figures to about the same extent and therefore does not lead to any shift in their mutual relationship.

The mortality figures for the other age groups of significance for cancer ventriculi, 30–49 years and over 80, do not change the impression that cancer ventriculi mortality is higher on the east than on the west side of the city (Table VI).

TABLE VI.—*Mortality from Cancer Ventriculi on the West and East Sides of Oslo*, 1930–50.

Deaths per year per 100,000 population December 1, 1937.

	West side.			East side.			Entire city.		
Age.	M.	F.	Total.	M.	F.	Total.	M.	F.	Total.
30–49	11	7	9	16	13	14	14	10	12
80 and over	696	527	568	775	837	813	731	655	680

Here it is clear that the difference between the east and west sides is relatively moderate for men, but very considerable for women. In the age-group 30–49 years the mortality is almost twice as high for women on the east side as on the west. It is characteristic that for both this age-group and for the group 80 years and over the mortality for men on the west side is lower than that for women on the east side, and on the east side the mortality for women is even higher than that for men in the oldest age-group. In other words it seems that on the east side women have been exposed to the irritants which may be responsible for higher mortality more than men, but that this difference is more pronounced for the oldest age-groups.

The object of dividing the Oslo population into east and west sides of the city was to obtain a population group with a generally good standard of living and one with a somewhat lower standard. There can, undoubtedly, be some question as to whether our division is the correct one. It is quite superficial. It can be demonstrated that income per consumer's unit as well as housing conditions were, on the average, better in that part of the city which we have called " west side " than in the part we have called " east side," but this does not afford a really sensitive

expression for the relationship between cancer ventriculi mortality and the social level in the period 1930–50 in Oslo.

The mortality figures for the various parishes will give a more differentiated picture (Table VII).

TABLE VII.—*Annual Mortality: Number of Deaths per 100,000 Population on December 1, 1937, from Cancer Ventriculi for the Age-groups 50–79 Years in the Period 1930–50 in the Individual Parishes of Oslo without Aker.*

Parish.	Men.	Women.	Total.
West side:			
Vår Frelser	167	110	134
Trefoldighet	177	117	140
Frogner	190	89	126
Uranienborg	152	103	120
Fagerborg	142	100	115
Gamle Aker	193	136	157
Markus	227	87	136
East side:			
Jakob	218	149	180
Sagene	223	147	180
Torshov	253	116	178
Paulus	229	141	177
Petrus	235	161	192
Grönland	293	207	247
Kampen	292	182	230
Gamlebyen	255	155	198
Vålerenga	214	174	192
Oslo total	214	130	164

In this table it is readily seen that the figures are relatively low for the most typical west side parishes and relatively high for the south-eastern parishes, and the tendency is convincingly obvious in the cartogram which has been made on the basis of these figures (Fig. 1). However, we still lack a more concrete characterization of the " social level " of the individual parishes. A characterization of this type would have to have a large number of components in order to be complete. But when only one single factor is used—and that should be permissible in this case—the average values for the amount paid in rent should probably be the most reliable.

On the basis of the municipal statistics of December 1, 1936, we have calculated weighted averages for seven groups of parishes. In order to obtain figures which are comparable with the figures for cancer ventriculi mortality, we had to make this grouping instead of giving the figures for each individual parish (Table VIII).

The negative correlation between cancer ventriculi mortality and average annual rent is not absolute in the sense that the mortality is highest in the group of parishes where the average annual rent is lowest, next highest in the group where the rent is next lowest, etc. The reason why the correlation is not complete may also be that rent is not an accurate characterization of living standards. At least there is no doubt that there is a negative correlation. We have drawn up the points in a diagram (Fig. 2) and indicated the curve which seems to illustrate the relationship between cancer ventriculi mortality and the social standard—characterized by the annual rent level—in each group of parishes. With so few points we have not calculated the mathematical line

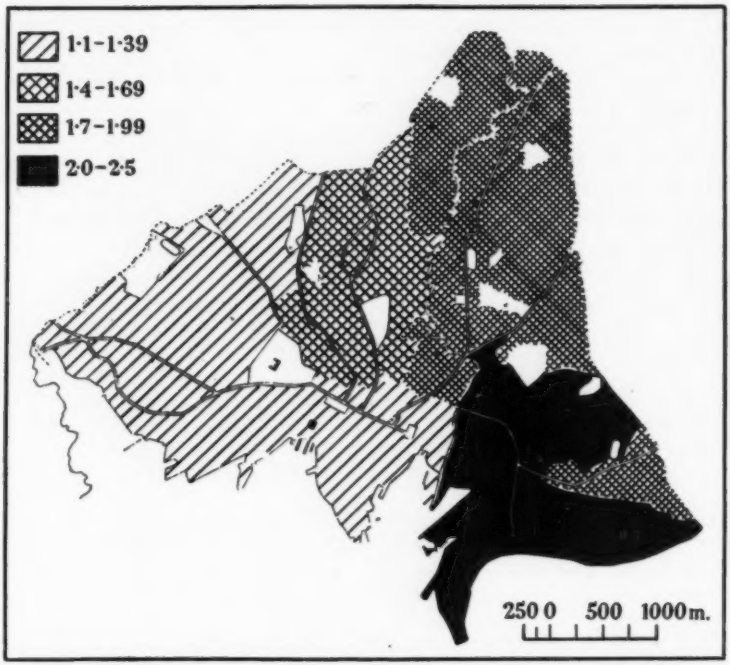

FIG. 1.—Annual mortality of cancer of the stomach—number of deaths per 100,000 population —for the age group 50–79 years. The period 1930–50 in Oslo.

of regression. As the figures stand the correlation does not follow a straight line, and the curve which is indicated is probably closer to the actual situation.

The conclusion again is that the material indicates that cancer ventriculi mortality on the average is higher among the poorer classes of the population in Oslo than among those better off. Before discussing the possible causes, on the assumption that the conclusion has a basis in fact, we shall examine in more

TABLE VIII.—*Annual Cancer Ventriculi Mortality for the Age-groups 50–79 Years in Seven Groups of Parishes and Annual Average House Rent in the same Groups of Parishes.*

Parishes.	Cancer ventriculi mortality. Men.	Women.	Total.	Average annual rent in 1936.
Vår Frelser	167	110	134	905 Crowns
Trefoldighet, Markus, Gamle Aker	197	115	145	923 „
Frogner, Uranienborg, Fagerborg	165	96	120	1,290 „
Sagene and Torshov	236	134	179	631 „
Paulus	229	141	177	534 „
Petrus	235	161	192	567 „
Grønland, Kampen, Gamlebyen, Jakob, Vålerenga	260	178	215	543 „
Whole city	214	130	164	765 „

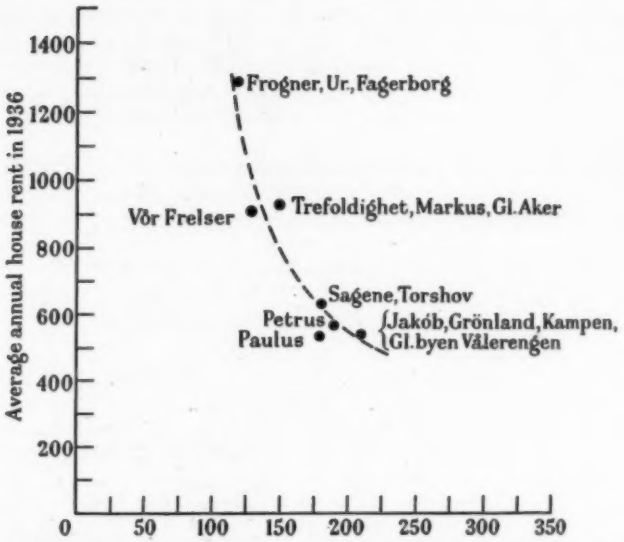

FIG. 2.—The relationship between cancer ventriculi mortality and the average house rent in Oslo 1930–50. The annual number of deaths from cancer of the stomach per 100,000 population for the age group 50–79 years.

detail the possible statistical errors that were mentioned above. Can incorrect diagnosis of cancer ventriculi have occurred systematically both in the country districts and in Oslo? Let us concentrate on Oslo to determine whether the statistics of the Bureau of Health can throw any light on this question.

According to the statistics of the Oslo Bureau of Health, a little more than 37 per cent of all cancer deaths in Oslo in 1930 were due to cancer of the stomach. In 1949 the corresponding figure was 22 per cent. Part of the explanation of the relative decrease in cancer ventriculi mortality as compared to total cancer mortality may be that cases which would previously have been recorded in the group which has always been the largest, namely cancer ventriculi, would now be recorded as cancer in other organs according to the more accurate present diagnostics.

Cancer of the liver, for example, may have been confused with cancer of the stomach. This would also explain why the difference between cancer ventriculi mortality in the cities and in the country districts is smaller in Sweden than in Norway and Denmark. According to Swedish practice, the nomenclature used in that country classifies cancer in both the stomach and the liver in the same group, so that an exact distinction between deaths from these two forms of cancer in the cities and in the country would not be so marked in the total statistics.

We have reviewed the statistics from the Oslo Bureau of Health and have found no marked tendency as regards the ratio between the number of deaths from cancer in the liver and gall bladder (cancer in these two organs was recorded in the same group until 1951 in the mortality statistics of the Bureau of Health) and

cancer of the stomach in the period 1930–50. The number of deaths from cancer in the liver and gall bladder comprise between 15 and 25 per cent (in one year, 1943, almost 30 per cent) of the number of deaths from cancer ventriculi, and even though there is a slight tendency toward an increase in the later part of the period the difference is too small to influence the proportion of cancer ventriculi mortality to total cancer mortality.

The number of deaths from cancer of the oesophagus is of a magnitude similar to deaths from cancer of the liver and gall bladder, but no definite tendency can be detected for the development of this form of cancer in the period 1930–50 either.

On the other hand, mortality from cancer of the colon shows a very characteristic tendency in the period 1930–50. If we divide the 21 years into 5-year periods, excluding 1950, the figures found are shown in Table IX.

TABLE IX.—*Mortality in Oslo (without Aker)*, 1930–50. (*Figures from Oslo Bureau of Health.*)

Period.	Cancer ventriculi. 1.	Cancer coli. 2.	2 as a percentage of 1.
1930–34	686	100	14·6
1935–39	715	153	21·4
1940–44	668	175	26·2
1945–49	658	232	35·3
1930–49	2,727	660	24·2

Here it is clearly seen that the number of deaths from cancer coli, in contrast to the number from cancer ventriculi, has increased considerably during the period 1930–50, and while the number of deaths from the former was only 14·6 per cent of the latter in 1930–34, it had increased to 35·3 per cent in 1945–49.

Nevertheless, the sum of deaths from these two forms of cancer has comprised a constantly decreasing proportion of total deaths from cancer during the period 1930–50. The increase in the number of deaths from cancer coli has not been sufficient to compensate the decrease in deaths from cancer ventriculi. The sum of the number of deaths from cancer of the stomach and of the colon represented not quite 35 per cent of all deaths from cancer during 1930–49, but the percentage proportion decreased from almost 39 in 1930–34 to about 33·5 in 1945–49.

Looking at the mortality from cancer coli (the statistics do not distinguish between cancer of the large and small intestine, but the majority of the cases are cancer of the large intestine, excluding rectum), we arrive at the figures in Table X.

TABLE X.—*Number of Deaths from Cancer Coli per* 100,000 *Living per year in Norway.*

Age-group.	Males. 1930–31.	Males. 1950–51.	Females. 1930–31.	Females. 1950–51.
30–39	2	1	1	0·5
40–49	3	4	4	3·0
50–59	11	12	17	14·0
60–69	39	29	25	29·0
70–79	52	85	60	84·0
80 years and more	65	164	45	207·0

It is easy to notice the marked rise in mortality for the age group above 70, and there is no doubt about the significant increase of cancer coli as cause of death in general occuring in the oldest group of the population. The corresponding figures for Oslo shows a similar picture (Table XI).

TABLE XI.—*Number of Deaths from Cancer Coli per* 100,000 *Living per Year in Oslo.*

Age-group.	Males.		Females.	
	1930–31.	1950–51.	1930–31.	1950–51.
30–39	7	3	—	1
40–49	—	6	6	4
50–59	4	18	19	15
60–69	66	47	37	32
70–79	63	121	58	99
80 years and more	77	205	59	247

Without a thorough revision of the material, that is, death certificates, charts, etc., it will be difficult to know if the statistics here express a true picture of the reality. It is of some interest to compare the development registered in the statistics of Norway during the period 1930 to 1950 to similar figures in England and Wales (Table XII).

TABLE XII.—*Death Rates per* 100,000 *Population in* 1931–35 *and* 1950–51 *for Cancer of the Stomach and Colon in England and Wales (Calculated from the Registrar-General's Statistical Reviews).*

Age-group.	Stomach.				Large intestine.*			
	Males.		Females.		Males.		Females.	
	1931–35.	1950–51.	1931–35.	1950–51.	1931–35.	1950–51.	1931–35.	1950–51.
35	12	9	7	5	4	5	6	5
45	42	36	23	16	16	12	18	16
55	107	98	64	45	52	37	49	39
65	219	210	151	124	138	114	126	94
75+	262	296	221	239	224	235	239	229

* Excluding rectum.

It is at once apparent that the tendency which appears so clearly in the Norwegian figures—fall in the mortality in cancer ventriculi and rise in the mortality from cancer of the large intestine—is not present in the same manner in England and Wales. In England and Wales, on the contrary, a fall in the mortality both from cancer ventriculi and coli in the period 1931 to 1951 is found, except in the age group 75+.

On the whole it would appear that improved diagnostic methods can hardly be the whole explanation of the decreasing significance of cancer ventriculi as a cause of death as compared to other forms of cancer in Oslo. If the figures represent a true tendency, it might be that the primary cause is to be found in the improvement in the standard of living which has undoubtedly taken place during the period 1930–50. If we assume that cancer ventriculi, more than other forms of cancer, is dependent on the " standard of living," the relative (and also absolute)

decrease in cancer ventriculi mortality is plausible. And it is in good agreement with the finding that cancer ventriculi mortality is higher on the east side than on the west.

In this connection it is interesting to notice that mortality from cancer coli—according to the information afforded from the statistics of the Oslo Bureau of Health—tends to be higher on the west side than on the east. When all deaths from cancer coli in the individual parishes in Oslo during 1930–50 are considered in relation to the native population aged 50 and over on December 1, 1937, we find 56 deaths per 100,000 on the west side as compared to 47 per 100,000 on the east side.

A statistical demonstration of the variation in cancer ventriculi mortality for the two parts of the city during the period 1930–50 would be desirable. Unfortunately our material does not provide figures which would make this possible and the mortality statistics of the Oslo Bureau of Health do not give sufficiently specified figures for such a calculation. Nevertheless, the tendency in development should appear from the following rather summary frequencies: Total number of deaths from cancer ventriculi (according to the Oslo Bureau of Health) in each part of the city in relation to the native population over 40 years of age in the respective parts of the city. The figures are shown in Table XIII.

TABLE XIII.—*Approximate Annual Mortality from Cancer Ventriculi for the Population of Oslo Aged 40 Years or More.*

	West side.			East side.			Entire city.		
Period.	M.	F.	Total.	M.	F.	Total.	M.	F.	Total.
1930–34	144	111	124	206	147	174	178	128	149
1935–39	130	109	17	181	129	153	158	118	135
1940–44	125	77	95	146	117	130	137	97	113
1945–49	103	67	81	128	100	113	117	83	97
Per cent decrease in mortality, 1930–34 to 1945–49	28·5	39·6	34·7	36·9	32·0	35·1	34·3	35·2	34·9

According to our hypothesis that the reduction in cancer ventriculi mortality is related to the improvement in living standard, one would expect that cancer ventriculi mortality would have decreased most markedly on the east side because it is reasonable to suppose that the population of the east side has benefited most from the general social improvement. But here again we know far too little of the changes in the consumer composition which have taken place in the various levels of the population. The propaganda for proper diet, for example, may have had just as much influence among the more well-to-do classes as among the poorer ones. At any rate it appears that the decrease in cancer ventriculi mortality has occurred almost parallel on the east and west sides of Oslo.

Our material may indicate that cancer ventriculi mortality varies with the standard of living, but the figures tell us nothing as to whether it is a lower standard of living in general—poorer housing, lower calorie consumption, harder work—which increases the disposition to cancer ventriculi, or whether one or several particular aspects of the lower standard of living are the decisive factors. The relation between cancer ventriculi mortality in the cities and in the country may

lead us to believe that certain aspects of living conditions, which were common for both the country and the east side populations during 1930–50, are significant. In any case, examination of several details will be necessary to provide a clue as to some of the causes.

To find a reasonable starting point for such investigations, it is necessary to begin with guesses. We shall mention a few things which might prove profitable fields for further investigation.

Housing conditions were probably the factor which characterized the difference in living standard most markedly during this period for the various population groups. Housing may have a direct influence, both physical and mental, and bad housing may also be the cause of unsatisfactory personal hygiene and health care.

Eating habits undoubtedly vary with the economic level. The—unfortunately all too limited—household investigations which have been made in Norway afford some information. They indicate that poor economy often leads to little variation in the diet, often more prepared foods, lower vegetable consumption and perhaps higher consumption of salt and spiced foods.

Whatever line is followed, great difficulties will be encountered in obtaining the necessary data. As the statistical apparatus becomes better developed, for example the statistics which aim to record private consumption, the problem will be facilitated. And as the calculations of national income require such detail statistics there is hope of improvement along these lines. At any rate the Cancer Registry will provide invaluable assistance in obtaining reliable material on cancer. There should be no doubt that this will afford the basis for profitable research in future years.

SUMMARY.

With a starting point in the hypothesis that cancer is environmentally determined we have compiled a material comprising deaths from cancer ventriculi in Oslo (without Aker) during the period 1930–50 from the records of the Oslo Bureau of Health.

The material seems to confirm that cancer ventriculi mortality in this period differed in various parts of the city and that it was, on the whole, higher in the eastern than in the western parts of Oslo. The relation between cancer ventriculi mortality and the economic level is also clear when the former is compared in relation to a single measure of the " social level " in seven groups of parishes in Oslo, namely the average annual house rent. The rule is that a high rent coincides with low cancer ventriculi mortality.

The figures show that cancer ventriculi mortality has decreased considerably from 1930 to 1950, this is true of the entire country, cities and country districts, and Oslo in particular.

On the basis of the figures for Oslo we discuss whether the decrease in mortality from cancer ventriculi as revealed by statistics can be due to a reduction in incorrect diagnosis during the course of the period, so that during the last part of the period, more than earlier, only the diagnostically reliable cases were included in the mortality statistics for cancer ventriculi. The Oslo Bureau of Health figures reveal a regular increase in both the absolute and relative number of deaths from cancer coli simultaneous with the decrease in cancer ventriculi mortality. However, the increase in the number of deaths from cancer coli is not sufficient to

compensate for the reduction in cancer ventriculi mortality. The number of deaths from cancer in the oesophagus, liver and gall bladder reveal no systematic tendency during this period. A confusion of different forms of cancer thus does not seem to provide the entire explanation of the decrease in cancer ventriculi mortality revealed by the statistics.

A Swedish investigation (Olinder, 1953) indicated that cancer ventriculi mortality—in contrast to mortality from cancer in other organs—was higher in the country districts than in the cities. Olinder's figures for 1946/47 indicate that the tendency is the same in Norway. Norwegian figures for 1930/31 and 1950/51, however, do not afford a basis for the contention of a significant difference between cancer ventriculi mortality in cities and country districts. For most other causes of death the mortality is highest in the cities, so cancer ventriculi differs from the others in this respect.

If there is a real connection between cancer ventriculi mortality and social conditions it would be reasonable to expect that the reduction in cancer ventriculi mortality from 1930 to 1950 would be greater among the east side than among the west side population in Oslo. The available figures do not indicate that this is so ; the reduction has been almost parallel in both parts of the city.

Finally there is a discussion of the individual factors which might be responsible for the tendency to excess mortality from cancer ventriculi among the poorer classes of the population of Oslo. Housing conditions, hygiene, eating habits and diet may be significant. Further investigations of these factors are necessary, however, in order to obtain more exact knowledge of their importance.

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THE PROGNOSIS IN HODGKIN'S DISEASE

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Hodgkin's disease is a disease of unproven aetiology, varied histological appearances and protean clinical manifestations. Exacerbations and remissions make the immediate outlook at first unpredictable, although there is rarely any doubt as to the ultimate outcome. These natural fluctuations make it difficult to estimate the value of any form of therapy, so much so that it has been suggested in the past that treatment has little real effect on the course of the disease. The main method of treatment at the moment is still X-ray therapy, but recent advances in the chemotherapeutic and hormonal control of cancer offer new lines of attack that may prove fruitful in the future. To judge the true value of any new agent of this type, a clear appreciation of the many factors influencing the natural history of the disease is of paramount importance. An agent offering some hope of absolute cure would largely remove this need, but unfortunately there is no sign that this will be available in the near future. We have therefore attempted to assess those factors which appear to affect the natural evolution of the process, and for this purpose have analysed all cases of Hodgkin's disease seen at the Middlesex Hospital in recent years.

MATERIAL.

We have included in this series all cases of Hodgkin's disease seen at the Middlesex Hospital between the years 1930 and 1952, provided that adequate histological material was available for examination. Biopsy specimens from many patients, particularly of those referred to the hospital during the war years, were no longer available. Over 100 cases have been excluded for this reason. The final series consisted of 227.

No selection of cases was made on their suitability for treatment. This would have affected our conclusions as to the outlook in this disease, and vitiated the object of the investigation. This series is not, therefore, strictly comparable with many previously published, where the mode of treatment is often one of the selection criteria. Many of our cases were far advanced when first seen, making any form of treatment ineffectual or impossible.

Although some of the patients were admitted and investigated in the general wards of the hospital, the majority were referred directly to the radiotherapy department, the diagnosis having been established elsewhere. The incidence of the disease as seen at this hospital is therefore completely unrelated to its true incidence amongst the populace at large.

CLINICAL FEATURES.

Extent of disease when first seen.

Hodgkin's disease presents with extremely varied clinical findings. The usual course of the disease is one of a gradual, progressive enlargement of the lymph nodes, at first limited in extent, later generalising and often finally invading structures other than lymphatic. In some cases there is widespread involvement almost from the onset, so much so, that the disease appears to be multifocal in origin. At this stage, constitutional symptoms are usual, and changes in the blood picture common.

The clinical staging of many tumours has sometimes been of great value, not only in the assessment of prognosis, but also often allowing a more rational approach to the treatment of the disease. The difficulties of clinical staging have been discussed recently (Smithers *et al.*, 1952) and with Hodgkin's disease the problem is particularly complicated. Clinical examination may suggest that the disease is limited to one area, whereas in actual fact there may already be widespread involvement of the whole body. Before deciding that the disease is probably limited in extent, we would prefer to know that X-ray examination of the chest is normal, that the patient is apyrexial and has no other constitutional symptoms, and that the blood count is within normal limits.

All cases in this series have been staged according to the extent of the disease when the diagnosis was first established. The system used resembles that of Peters (1950), differing mainly in our definition of the third stage of widespread disease (Table I). We consider that the presence of constitutional symptoms, in

TABLE I.—*Staging of Hodgkin's Disease.*

Stage I	Lymph node involvement in only one main group.
Stage II	Lymph node involvement in 2 or more adjacent groups in either upper or lower half of body.
Stage III	(*a*) Generalised lymph node involvement. (*b*) Constitutional manifestations for which no other reasonable cause can be found. (*c*) Disease apparently limited to retroperitoneal lymph nodes. (*d*) Involvement of structures other than lymphatic.

the absence of any other reasonable explanation, is certain evidence that the disease has generalised. Unexplained pyrexia, pruritus, night sweats, and a blood count showing a low haemoglobin, and a high polymorphonuclear count all suggest advanced disease. A leucopenia, often with depression of red cell and platelet elements is not infrequently found in the more fulminating cases.

All cases presenting with disease apparently limited to the retroperitoneal lymph nodes, have also been included in the third stage, even in the absence of constitutional symptoms, although at first sight, it would appear justifiable to include them in Stage I. Not only was the tempo of these cases rapid, but also the site made adequate irradiation difficult. Only three of our cases fall into this group. In each, the presenting symptom was abdominal pain for which laparotomy was performed. Survival after the diagnosis had been established were 12, 22, and 26 months. The outlook in this type of case therefore fully justifies

its inclusion in the third stage. Most patients with retroperitoneal lymph glands have severe constitutional symptoms or generalised disease and therefore fall automatically into the advanced stage.

Invasion of extra-lymphatic structures may either occur late, in longstanding, relatively benign cases, or occasionally as an early complication in the more acute type of case. In either case the ultimate outlook is poor. Very rarely the disease process apparently arises in, and is limited to, some unusual site. For example, Hodgkin's disease macroscopically localised to the stomach has been found at laparotomy, with no obvious evidence of spread elsewhere. This type of case could be classed as early, and five-year survivals are obtainable if adequate resection or irradiation is possible (Slaughter and Craver, 1942).

Cases of Hodgkins' disease apparently arising in the skin have not been included in our staging. Although only one organ is involved, the disease may cover large areas of the body. In spite of this, the outlook may be very good, until lymph node involvement finally develops. The disease then usually progresses rapidly. Three of our cases presented originally in the skin, and these cases have, therefore, been excluded from those tables including staged cases. Cases developing skin invasion as the disease advances are, of course, included in the third stage.

Examination of our results show that the outlook is very different in the three stages (Table II). Fifty-nine per cent and 60 per cent respectively of the Stage I

TABLE II.—*Hodgkin's Disease. Stage, Sex and Survival.*

		All cases 1930–1952.		All cases 1930–1947.		5-year survivals.		
		Total.	Percentage of total.	Total.	Percentage of total.	Total.	Percentage of total.	Percentage 5-year survival.
Stage I	Male	23	53	19	59	10	53	52
	Female	20	47	13	41	9	47	69
	Both sexes	43	100	32	100	19	100	59
Stage II	Male	31	54	24	51	12	43	50
	Female	26	46	23	49	16	57	70
	Both sexes	57	100	47	100	28	100	60
Stage III	Male	84	68	64	70	1	33	2
	Female	40	32	28	30	2	66	7
	Both sexes	124	100	92	100	3	100	3
All stages	Male	138	62	107	63	23	46	21
	Female	86	38	64	37	27	54	42
	Females 20–40	—	—	48	28	23	46	48
	Both sexes	224	100	171	100	50	100	29

Three primary skin cases have been excluded. All percentages calculated to nearest whole number.

and Stage II cases survive 5 years, compared with only 3 per cent of the Stage III cases. Of the total of 124 Stage III cases, 8 died in under 4 weeks, 62, or 50 per cent, died in under 1 year, and 107 or 86 per cent by the third year.

Fifteen cases of the entire series have survived for 10 years or longer. Of these, 6 were in Stage I, 9 were in Stage II and none in Stage III when first seen.

Duration of disease when first seen.

It is usually accepted that the outlook is better in malignant disease if the case is not too advanced when the patient is first seen. But whatever the stage reached by the disease, the time taken to reach that stage may be accepted as indicating its general tempo, and the prognosis modified accordingly.

In our experience, Hodgkin's disease cannot be fitted into this general pattern. It is often extremely difficult to assess the true duration of some cases, as constitutional symptoms due to other diseases may have preceded its onset. Superficial lymph nodes may be unnoticed by the patient for long periods, mediastinal involvement may be detected only on X-ray examination of the chest, and involved upper abdominal glands are notoriously difficult to detect at an early stage. For the purpose of this analysis we have assumed that symptoms or signs fitting into the diagnosis, and not satisfactorily explained by any other means, are due to Hodgkin's disease, and have dated the onset accordingly.

In each stage, there are cases showing considerable variation in the duration of the disease before the diagnosis was established. One Stage III patient noticed a gradual enlargement of lymph glands for just under 9 years and died 2 months after the diagnosis had been established. Another had a generalised enlargement of glands for only 1 month before a biopsy was taken, and yet he survived for 4½ years. In 2 cases in Stage II the disease had been present for just under 2 years before the diagnosis was established. The first case died 10 months later and the second is still alive to-day, 19 years later, having had no further trouble. The average duration before diagnosis of all cases in Stages I, II and III respectively, was 11, 11 and 10 months. The total average duration before diagnosis of all cases not surviving 5 years was 10 months, and of all cases surviving longer than 5 years, 14 months. This is not a very marked difference, and we do not consider that the duration of the disease process before the diagnosis is established is of any great value in assessing the prognosis. Of far greater importance is the stage the disease has reached when the patient is first seen.

Age at the onset of the disease.

The age of the patients at the onset of the disease in this series varied between 2 and 78 years. The age distribution in the two sexes is shown on the accompanying figure (Fig. 1). Jackson and Parker (1947) found that the prognosis in their series was particularly poor in the very young and the very old and we agree with their findings. Analysis of our series shows that the outlook is best in the third and fourth decades, and this was particularly marked amongst the female cases.

Sex.

The total series of 227 cases consisted of 139 males and 88 females, a proportion similar to that usually reported by other authors.

Our results confirm the previously suggested view that the outlook is less favourable in the male. Of all cases seen before 1948, 50, or 29 per cent, survived 5 years. Of these 50, 54 per cent. were female and 46 per cent men, which is a reversal of the initial sex incidence. Equally striking is the 48 per cent 5-year survival rate amongst females in the third and fourth decades—a figure appreciably greater than that of the general survival rate (Table II). It is true that 61 per cent of the males presented in Stage III, whereas only 46 per cent of the females

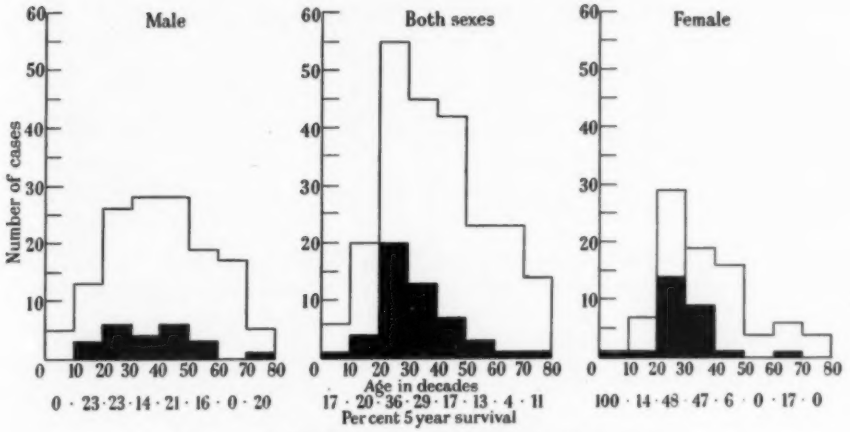


FIG. 1.—Hodgkin's disease. Age of onset and survival.
All cases. 5-year survivors.

were as far advanced when first seen. However, this cannot explain the difference in survival rates, as in each individual stage, this sex difference was maintained.

PATHOLOGY.

The histological material of the patients forming this series has been examined and classified into three sub-groups, as proposed by Jackson and Parker (1947). These authors divide their cases of Hodgkin's disease into " paragranuloma ", " granuloma " and " sarcoma ", according to the cellular characteristics displayed in the lymph nodes.

In their series of cases there were 41 patients with paragranuloma. The prognosis of this sub-group appears excellent, as 15 remain alive and well 5 years or more after diagnosis, but 7 eventually progressed to the " granulomatous " form of the disease.

There were 237 cases of " Hodgkin's granuloma " with an average prognosis of $2\frac{1}{2}$ years, and 51 patients with " Hodgkin's sarcoma ", the majority of whom were dead within a year of diagnosis.

According to these authors it would appear that each type of Hodgkin's disease has a clearly distinguishable behaviour pattern and prognosis.

The prognosis of the sub-groups of the present series of Hodgkin's disease does not entirely substantiate these findings, and, for reasons stated later, we prefer to use the terms Hodgkin's disease Grade 1, 2 and 3 instead of the three named sub-groups of Jackson and Parker.

Hodgkin's disease, Grade 1.

In this series of 227 patients, the histology of 8 (4 per cent) showed the cellular pattern corresponding to " paragranuloma ". The clinical features of this grade are summarised in Table III. The average age at onset was 28 years. Six cases

TABLE III.—*Clinical Features. Hodgkin's Disease, Grade* 1.

Case No.	Age.	Sex.	Stage.	Survival.	
E72	12	M	II	19 years.	Alive
M142	32	M	I	2 „	„
B227	28	M	I	16 „	Dead. Generalised
D62	51	F	I	4 „	Alive. Recurred
D71	51	M	I	10 „	„ „
O'G159	13	M	I	5 „	„ „
M144	26	F	I	11 „	Dead. Generalised
M154	24	M	II	2 „	Alive

showed cervical node involvement only, 2 cases had both cervical and axillary disease, and there were no examples of generalised disease at the time of presentation.

All the patients were treated by irradiation and 6 remain alive and well from 2 to 19 years later. Two patients died in the 11th and 16th year with generalised Hodgkin's disease. In one case autopsy showed that transformation had occurred to the Grade 2 form. No autopsy was performed on the other fatal case.

Hodgkin's disease Grade 2.

Two hundred and two patients (88 per cent) showed the histological picture corresponding to the Hodgkin's " granuloma " of Jackson and Parker (1947).

The average age at the time of onset of the disease varied from 2 to 78 years, with an average of 35 years. There were 121 male and 81 female patients.

Table V summarises the prognosis of this grade of Hodgkin's disease. Of the 156 possible 5-year survivals, 111 were dead within 5 years of diagnosis. This gives a survival figure of 29 per cent. If this survival figure is assessed according to the stage of the disease at the time of presentation it will be seen that of the 72 patients in Stages I and II, 42 remained alive at the end of 5 years, a survival rate of 58 per cent. Of the 84 cases in Stage III only 3 survived 5 years, a survival rate of 4 per cent.

One patient survived 16 years, while 11 survived 10 years. A number of cases have, however, succumbed to the disease within a few months of treatment and the histological pictures in these cases differ in no recognisable way from the more successfully treated patients of the same grade.

Hodgkin's disease, Grade 3.

There were 17 examples of the cellular type of disease (8 per cent) corresponding to the Hodgkin's " sarcoma " of Jackson and Parker. The clinical features of this group are summarised in Table IV.

The average age at onset was 44 years, and this grade appears to occur in an older age group than Grades 1 and 2.

From Table IV it will be seen that 16 of the 17 cases are dead and survival periods ranged from 1 month to 4½ years. One death occurred 4½ years after diagnosis, 1 at 3 years and 2 at 2 years ; thus the disease, although fatal, does not necessarily kill rapidly.

Another feature of the Hodgkin's Grade 3 is the high percentage of Stage III cases, so that the survival figures remain comparable to the other varieties of Hodgkin's disease of a similar clinical staging. Nevertheless, as Table V shows,

TABLE IV.—*Clinical Features. Hodgkin's Disease, Grade* 3.

Case No.	Age.	Sex.	Stage.	Survival.	
C60	78	M	III	5 months.	Dead
B18	63	M	III	2 years.	,,
B33	61	M	III	1 month.	,,
H103	26	F	II	1½ years.	,,
L136	30	M	III	1 year.	,,
M145	28	F	III	7 months.	,,
S185	61	M	III	1 year.	Alive
H115	49	M	III	1 ,,	Dead
C59	19	M	III	6 months.	,,
W203	57	M	I	4 ,,	,,
S180	70	F	III	13 ,,	,,
M138	42	M	III	4½ years.	,,
E78	38	M	II	4 months.	,,
T201	59	M	III	3 years.	,,
S184	44	F	III	2 ,,	,,
B23	9	M	III	2 months.	,,
H117	14	F	III	4 ,,	,,

TABLE V.—*Hodgkin's Disease. Grade, Stage and Survival.*

Grade.	Stage.	Possible 5-year survivors.	5-year survivors.	Percentage 5-year survivals.
Grade 1	I	4	4	100
	II	1	1	100
	III	0	0	0
	All stages	5	5	100
Grade 2	I	27	15	55
	II	45	27	60
	III	84	3	4
	All stages	156	45	29
Grade 3	I	1	0	0
	II	1	0	0
	III	8	0	0
	All stages	10	0	0
All grades	I	32	19	59
	II	47	28	60
	III	92	3	3
	All stages	171	50	29

of the 10 possible 5-year survivors not one patient in this small series attained that figure.

DISCUSSION.

The purpose of the pathological section of this article is to assess the part that histology can play in evaluating the prognosis of a given case of Hodgkin's disease. Jackson and Parker (1947) claimed a reasonably accurate assessment in their series of cases, but we have been unable to substantiate the value of this work in its entirety.

Hodgkin's disease, Grade 1.

This is clearly a histological entity which is diagnosable on lymph node biopsy without knowledge of the clinical condition. Harrison (1952) gives an excellent

description of the pathological features found in the lymph nodes in this disease to which we have nothing to add (Fig. 2 and 3).

In view of the facts that local recurrence may follow treatment and the disease may eventually change its characteristics, becoming generalised and fatal after a period of years, it seems more realistic to regard this grade as a more slowly progressive form of Hodgkin's disease without being a different disease entity. The terms " paragranuloma ", " lympho-reticular medullary reticulosis " (Robb-Smith, 1947) and " reticular lymphoma " (Lumb, 1954) suggest a different disease process from the ordinarily accepted meaning of Hodgkin's disease, and were presumably intended to do so. The title " Benign Hodgkin's Disease " conferred upon it by Harrison (1952) appears particularly inappropriate for a condition that may be fatal.

We prefer to denote this recognisable variety of Hodgkin's disease by the non-committal term, Hodgkin's disease, Grade 1. This implies a lesion with the clinical and histological characteristics of Hodgkin's disease, but of a lower grade malignancy with potentialities for eventual generalisation in some cases.

Hodgkin's disease, Grade 2.

As in Jackson and Parker's series this forms the bulk of the cases and histologically the cellular appearances of the lymph nodes are those generally accepted and described in every text-book of pathology (Fig. 4 and 5).

The prognosis in this type of disease is extremely variable and appears to be modified by treatment (Gilbert, 1939). Cases with a virtually identical histological pattern may live for 15 years, or, conversely, die within a few months of diagnosis. The prognosis appears more closely related to the clinical staging and although an average prognosis of 29 per cent 5-year survival can be given for the whole group the histological pattern has no part to play in the accurate prognostic assessment of this grade of Hodgkin's disease.

The name, " Hodgkin's granuloma ", seems inappropriate as implying a non-neoplastic, chronic inflammatory aetiology; an assumption which is not very realistic for such a widely disseminated and invariably fatal disease.

A more logical approach to nomenclature is Hodgkin's disease, Grade 2. This implies a disease process of unknown aetiology showing the microscopical appearances of accepted Hodgkin's tissue and demarcating it from the Grade 1 and Grade 3 counterparts.

EXPLANATION OF PLATE.

FIG. 2.—*Hodgkin's Disease, Grade* 1. Showing loss of the normal architecture of the lymph node due to lymphocytes and conspicuous giant cells. × 100.

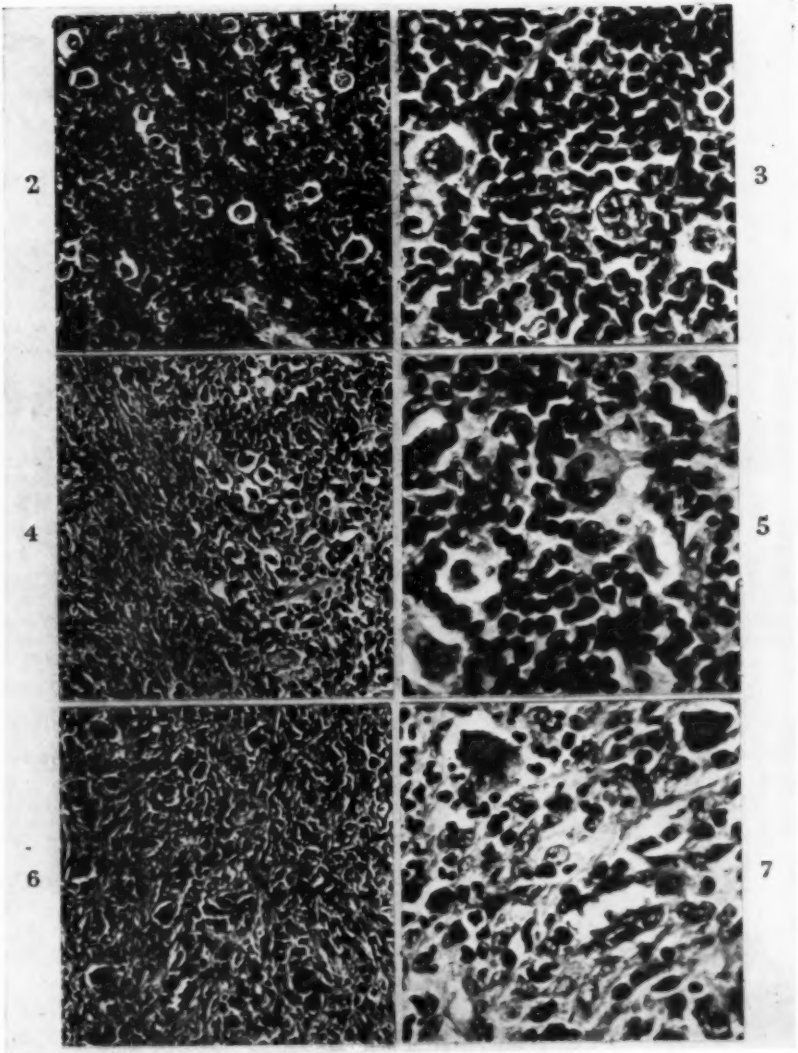
FIG. 3.—*Hodgkin's Disease, Grade* 1. Showing the giant cells embedded in a predominantly lymphocytic stroma. × 500.

FIG. 4.—*Hodgkin's Disease, Grade* 2. Showing loss of the normal architecture of the lymph node with areas of fibrous tissue and cellular foci containing giant cells. × 100.

FIG. 5.—*Hodgkin's Disease, Grade* 2. Showing the cellular pleomorphism with giant cells, reticulo-endothelial cells, polymorphonuclear leucocytes. × 500.

FIG. 6.—*Hodgkin's Disease, Grade* 3. Showing the loss of the normal architecture with a marked cellularity and many giant cells. × 100.

FIG. 7.—*Hodgkin's Disease, Grade* 3. Showing the pleomorphism of the cells with giant cells, conspicuous reticulo-endothelial overgrowth and mitotic activity. × 500.



Jelliffe and Thomson.

Hodgkin's disease, Grade 3.

This grade of Hodgkin's disease has ill-defined limits ; at one end of the scale it merges into the Grade 2 type and at the other end with reticulum cell sarcoma. As Jackson and Parker (1947) state, the one diagnostic feature is the presence of a variable number of Dorothy Reed giant cells in an actively growing reticulo-endothelial cell proliferation. To add to the confusion, Jackson and Parker's photomicrographs are not diagnostically informative and Custer and Bernhard (1948) regard the Hodgkin's sarcoma as virtually synonymous with reticulum cell sarcoma.

However, there does appear to be a small number of cases in this series showing the characteristic cellular pleomorphism of Hodgkin's disease containing the diagnostic giant cells. In addition, there is an abnormally active reticulo-endothelial cell overgrowth with frequent mitotic figures and local areas of necrosis. The appearances resemble an actively growing tumour and the fibrous tissue, so prominent in the Grade 2 counterpart, is scanty. This type is called Hodgkin's disease, Grade 3, to denote a pathological process at the more cellular and malignant end of the scale of Hodgkin's disease (Fig. 6 and 7). In this sub-group the tempo of the disease is extremely rapid, and 12 of the 16 deaths occurred within 18 months of diagnosis. No case survived for more than $4\frac{1}{2}$ years.

Other features are worthy of comment in this series as a whole. There is an apparent therapeutic success in female patients with Hodgkin's disease restricted to the mediastinum and cervical regions. This success may be due to confusion of the histological appearances with the granulomatous form of malignant thymoma (Eisenberg and Sahyoun, 1950 ; Thomson, 1955).

In 29 cases, serial biopsies were available. In 11 cases an increased cellularity of the Hodgkin's tissue was detectable in the subsequent pathological material as the disease progressed. We found no examples of complete cellular alteration necessitating a different diagnosis, as did Custer and Bernhard (1948).

TREATMENT.

Bearing in mind the many factors influencing the course of Hodgkin's disease, it is difficult to assess the true value of any form of treatment. Of the 227 patients in this series, 14 were untreated ; 5 presented in Stage I, and 9 in Stage III. The average survival after diagnosis was under 4 months in the last group, and 41 months in the first. One Stage I case survived for just over 5 years. These cases cannot be considered an adequate control series. Firstly, the number was small and the overall figures must be unduly influenced by a few good or bad results. Secondly, there was definite selection of the Stage III cases, 4 being considered too advanced for any attempt at treatment. They do, however, emphasize again the totally different outlook in these two stages.

Surgical resection of the diseased area has been recommended as the ideal method of treatment, when technically possible (Baker and Mann, 1940). Slaughter and Craver (1942) quote 5 patients alive and well, 5, 6, 8, 11 and 11 years after removal of the affected lymph nodes by block dissection. Three of our patients were treated in this manner, but in each case the operation was followed by X-ray therapy. One patient had developed lymph glands in the adjacent supra-clavicular triangle by the time X-ray therapy was commenced, a few weeks after an apparently successful dissection. One case has survived for 5 years.

There does not appear to be much place for this method of treatment. If the disease is genuinely limited to one area, its extreme radio-sensitivity makes radical X-ray therapy the ideal method for general purposes. In very exceptional cases the disease may be so localised that complete resection is possible, and occasionally it may arise at some unusual site, such as the stomach, where adequate irradiation is difficult. Resection in this type of case would offer the only real hope of long survival (Slaughter and Craver, 1942).

Irradiation has been the main method of treatment in the majority of our cases. A few of the earlier patients were exposed to radium, either in the form of a surface applicator, teleradium, or needle implant. Since the earlier years all irradiation has been by means of 170–250 kv. X-rays, 1–3·8 mm. Cu h.v.l. Throughout various centres, there is considerable difference of opinion as to the most effective methods of treatment (Medinger and Craver, 1942; Peters, 1950; Levitt, 1952; Nice and Stenstrom, 1954). We have analysed all cases treated initially by deep X-rays, in an attempt to assess the dose necessary to ensure local cure of involved nodes. Excluding all Stage III cases, the remainder can be placed in three main groups. The first includes those cases with complete disappearance of the treated nodes, and no recurrence, locally or elsewhere, the patient known to have had no further trouble, for a period of 5 years or longer (Table VI). The

TABLE VI.—*Cases Without Recurrence after First Treatment.*

Stage.	X-ray dosage (t.d.).	Survival (years).
I	2000 r	6½
I	2100 r	5
I	3000 r	8
II	3000 r	12
II	3500 r	19
II	4000 r	14
II	3730 r	8
II	6400 r	11

t.d.: Tumour dose.

second includes all cases where disappearance of the nodes was followed by a first recurrence at the previously treated site (Table VII). By doing so, those cases are excluded where apparently local recurrence could have been caused by retrograde spread from adjacent involved areas. All cases in the second group received a dose of at least 2000 r and 6 received at least 3000 r. Amongst those cases with the first recurrence at the previously treated site, 13 were given 2200 r or less, and none received more than 3000 r. The total number of cases is small, but this suggests that complete local resolution is probable above a dose of 3000 r, while a local recurrence is more likely if less than this amount of X-ray therapy is given. From this it follows that reasonably localised disease should be treated to a level of at least 3500 r over 3½ to 4 weeks, provided that this is technically possible, if an attempt is to be made at a radical cure.

Wide field irradiation may be necessary in the later stages of the disease. Large volumes of body tissue cannot be raised to such dose levels with safety, and palliation of a more temporary nature is the most that can be expected.

The cytotoxic poisons have been used at the Middlesex Hospital with increasing frequency since their introduction by Wilkinson (Wilkinson and Fletcher, 1947).

TABLE VII.—*Cases with First Recurrence at Site of Previous Irradiation.*

Stage.	X-ray dosage (t.d.).	Recurrence.
I	400 r	3 years
I	1000 r	4 ,,
I	2000 r	1 year
I	3000 r	1½ years
I	3000 r	1 year
II	1000 r	2½ years
II	1000 r	5 ,,
II	1050 r	6 months
II	1150 r	4 ,,
II	1500 r	3 ,,
II	1800 r	6 ,,
II	1800 r	7 ,,
II	2200 r	6 ,,
II	2200 r	3 years
II	2400 r	10 months
II	3000 r	3 years

t.d.: Tumour dose.

The majority of cases have been given nitrogen mustard, often in repeated courses. Their use has been well reviewed recently by Beatty and Howells (1954). There does not appear to be any evidence available that these agents are preferable to X-rays in the treatment of localised Hodgkin's disease. Almost all our cases treated with these agents had widespread disease, and it is therefore hardly surprising that responses have been short-lived. Nitrogen mustard was given as the first method of treatment in only 5 cases. Survivals after treatment were 34, 33, 23, 5 and 2 months. This is not a fair comparison with those cases treated by X-rays, as 4 of the 5 were in Stage III. The one early case, a girl of 23, with disease limited to the cervical glands on one side, had a remission of 9 months following which the disease progressed rapidly. The danger of severe and possibly fatal depression of all the bone marrow elements is common to both the cytotoxic poisons and to wide field irradiation. There does not appear to be any logical reason for their use in apparently localised disease, when local irradiation can be given to a high dose level without any severe general body effects. Even when indicated by widespread disease, care is needed in their administration, but provided adequate control is maintained they have an important place in the management of Hodgkin's disease.

COMPLICATIONS.

Hodgkin's disease is almost invariably fatal. The exact cause of death may be difficult to ascertain; and was often so in this series. It was frequently difficult to obtain precise clinical details or post-mortem findings, as many of the patients died at home or in other institutions. In some cases the disease was so widespread at autopsy that the exact cause of death could not be decided. In spite of this, a review of the common complications occurring in this series revealed some points of importance. It is probable that in some cases their earlier recognition and more adequate treatment might have led to the prolongation of useful life.

Pregnancy.—As with tuberculosis, it has often been held that pregnancy is contra-indicated in Hodgkin's disease, in that it is frequently followed by an exacerbation of the disease process. Eight patients in this series became pregnant. We found no evidence that this occurrence altered an any way the tempo of the disease, but no reasonable conclusions can be drawn from such a small series. Stewart and Munro (1952) have reviewed the subject. After analysing 134 reported cases, they conclude that pregnancy has no predictable effect on the course of the disease.

Blood abnormalities occurred frequently as the disease progressed. An anaemia was the rule in the more advanced cases. This was not usually of a severe degree, and was correctable by transfusion and by treatment of the underlying disease. In some cases it became a major problem, and repeated transfusions failed to raise the haemoglobin above dangerously low levels. None of these cases had any obvious blood loss from any site. Two patients developed a haemolytic anaemia ; one as a terminal complication for which nothing could be done, while the other responded well to cortisone, followed by splenectomy.

Leucopenia and thrombocytopenia, often associated with a low haemoglobin level, were frequently found at a late stage in the disease. The majority of cases appear to have followed either invasion of the bone marrow or prolonged treatment with the cytotoxic poisons or X-rays. The leucopenia is often a deciding factor, preventing further treatment and predisposing the victim to secondary infections. It is therefore important to conserve the bone marrow for as long as possible, and it is probably advisable to avoid the use of cytotoxic poisons and wide field irradiation, unless they are indicated by generalised disease.

An eosinophilia is found in under 10 per cent of all cases of Hodgkin's disease. In our experience an increase of the eosinophil count above the normal range was found only when widespread disease was present, when there was macroscopic invasion of the skin, or when the patient complained of pruritus. We therefore consider that an eosinophilia, if present, suggests that the disease is widespread. The only exception to this was seen in those cases with skin invasion as the primary manifestation. As has been mentioned previously, the outlook in this type of case appears to be extremely variable, and an eosinophilia cannot then be considered a sign of poor prognosis.

Intrathoracic spread is extremely common, occurring in the majority of cases. Hilar gland enlargement is, of course, the commonest form, occurring at some stage in two-thirds of our cases. This finding does not appear to influence the prognosis adversely. Twenty-nine of the cases in Stage II when first seen had hilar gland enlargement on X-ray examination of the chest. Sixteen or 55 per cent, of these cases survived for more than 5 years Only one of the 227 patients developed clinical signs of superior vena caval obstruction.

The presence of parenchymal pulmonary involvement is, however, of more serious import. Diffuse infiltration of the lung fields was found commonly in the late stages of the disease, and 5 patients developed discrete rounded intrapulmonary opacities. No case with parenchymal infiltration responded satisfactorily to treatment. One patient died of haemorrhage from a main branch of the right pulmonary artery, which ruptured into the centre of a necrotic mass of Hodgkin's tissue.

Intra-abdominal spread involving the retroperitoneal glands occurs at some stage in the majority of patients with Hodgkin's disease.

In the present series, retroperitoneal gland involvement was found, either clinically, or at autopsy in over one half of all cases. Once this complication occurred the outlook became gloomy, as involvement of the renal tract and hepatic system were common sequelae. Probably a number of the cases of so-called "Hodgkin's cachexia" were unrecognised cases of uraemia. In addition the commonest site of extra-dural deposits of Hodgkin's tissue is in the lower dorsal and upper lumbar spine, presumably from direct spread backward from the retroperitoneal tissues. Thirteen of the 16 cases of paraplegia in this series occurred in this region.

The difficulties of treating a large mass of glands in the upper abdomen have already been mentioned, and diagnosis at an earlier stage may be extremely difficult. There may be nothing to feel on abdominal examination, but a history of recurrent attacks of upper abdominal pain, often radiating through to the middle of the back and waking the patient in the small hours of the morning is very suggestive of retroperitoneal spread. Intravenous pyelography may be a useful confirmatory investigation, demonstrating displacement of the kidneys or ureters. If no other cause can be found, irradiation of the affected area usually leads to a rapid improvement of symptoms, thus confirming the provisional diagnosis.

Nervous system involvement is a common complication of Hodgkin's disease.

In this series, 8 patients developed actual invasion of peripheral nerves, including the brachial plexus, cervical sympathetic chain, and oculomotor and recurrent laryngeal nerves. Two patients developed a multiple peripheral neuritis. All cases were advanced, and the ultimate prognosis was unaffected by these complications.

Coma was known to occur as a terminal event in at least 17 of the patients, associated in some cases with convulsions and preceded by mental disturbances. In some of these cases the clinical appearance of the patient suggested the possibility of the development of intra-cerebral deposits. In fact no such invasion was found in any case at post-mortem examination. This result is not unexpected, as there are only four acceptable examples of brain invasion in the world literature reviewed recently by Fein and Newill (1954). One patient was found at post-mortem examination to have an intra-cranial extradural deposit of the disease.

Extradural deposits with paraplegia developed in 16 cases, 9 without obvious change in the vertebral column on X-ray examination. The importance of the early recognition and treatment of this complication is well illustrated by one patient who lived a normal life for 4 years following laminectomy and irradiation of the affected cord segment, until further generalisation of the disease occurred. Paraplegia may develop at any state of the disease, and in 2 of the 16 cases it was the presenting symptom. This subject is well reviewed by Smith and Stenstrom (1948).

Herpes zoster occurred in 27 patients. This is a significant complication, as it was followed within 3 months by an exacerbation of the underlying Hodgkin's disease in all except 2 cases.

Secondary infection.—This complication is probably of less importance following the introduction of the antibiotics, but it is always potentially dangerous in the later stages of the disease, when the polymorphonuclear count is low.

Tuberculosis has long been associated with Hodgkin's disease, so much so that a common cause has often been postulated. Seven patients in this series devel-

oped tuberculous lesions. This does not appear to be excessively frequent in a disease which may be both debilitating and long-standing. Part, at any rate, of this traditional association may be due to misdiagnosis. Five case of Hodgkin's disease were thought, on clinical examination, to have tuberculous glands of the neck, and were treated as such for variable periods before the correct diagnosis was finally made by biopsy. One of these patients was considered tuberculous for over 4 years, one of which was spent in a sanatorium. In the late stages of the disease, pulmonary infiltration with Hodgkin's tissue is very common, giving X-ray appearances that may be indistinguishable from those seen in pulmonary tuberculosis.

In all our tuberculous patients the underlying Hodgkin's disease was already advanced and the outlook, therefore, extremely poor. Four deaths were undoubtedly directly due to tuberculosis. Presumably, with modern antibiotics, the prognosis is now better in this type of case.

Complications unrelated to Hodgkin's disease.

It is very easy to miss the development of a new disease in the course of a chronic illness. Additional complications may be attributed to the original complaint with fatal results. Three of our patients developed a perforation of a duodenal ulcer. Two were seen elsewhere, the pain was attributed to Hodgkin's disease and the correct diagnosis only established at post-mortem examination. One case was recognised and successfully dealt with. A proven peptic ulcer occurred in 7 patients, and was a constant source of difficulty in diagnosis, the pain being confused with that found with retro-peritoneal gland involvement. This high incidence may be attributed to the constant nervous tension under which the majority of these unfortunate patients must live.

CONCLUSIONS.

The relative merits of the different forms of treatment in Hodgkin's disease cannot be evaluated successfully unless the cases under review are strictly comparable. An analysis of this series suggests that many factors affecting both the natural history and prognosis of the disease can be clearly recognised.

The important features which determine the eventual prognosis include the stage reached by the disease at the time of presentation, the histological appearances, the age at onset and the sex of the patient. In our experience the duration of the disease before treatment has proved of little prognostic value, as it is often impossible to assess this factor accurately.

Staging of the disease at the time of the initial clinical examination has proved of considerable value Of the patients placed in Stages I and II about 50 per cent are alive at 5 years, while those in Stage III at the time of presentation very rarely survive this period. During the earlier years covered by this series some of the patients included in Stages I and II were insufficiently investigated to exclude completely early generalisation of the disease. The ensuing short survival of many of these patients suggests that, with more searching clinical, radiological and haematological investigation, some of these cases would have been more accurately placed in the Stage III category of the disease. If this assumption is correct the outlook in the Stages I and II would compare even more favourably with Stage III.

There does appear to be some difference in the prognosis of the various age-groups and between the two sexes. An analysis of the results suggests that the best survival rate is found in young women in the third and fourth decades. Unfortunately, the number of patients in this series is small and further subdivision of the cases into groups according to the age and sex renders more detailed analysis of doubtful significance.

Histological examination of biopsy material is essential as the sole method of establishing the specific diagnosis. In the absence of histological proof both the diagnosis and, therefore, the prognosis remain entirely conjectural. It is possible to recognise three histological grades in Hodgkin's disease, each with a significantly differing average prognosis. All the patients with a Grade 1 microscopical appearance were alive 5 years after diagnosis, while no histologically Grade 3 patient survived this period. Taking each grade separately, it was not possible to decide on histological grounds alone which individual case would fare better or worse than the average within that grade.

Eighty-eight per cent of the cases of this series have a Grade 2 type of cellular picture, and there is no recognisable histological distinction between those patients who survive for 15 years and those who succumb to the disease in as many weeks. This is the major limitation of the histological grading of Hodgkin's disease. However, it is extremely valuable in assessing the prognosis as a whole to recognise both the Grade 1 and Grade 3 varieties of the disease, although these account for only a small percentage of the series.

Although many of the complications of Hodgkin's disease, such as parenchymal lung involvement and retroperitoneal spread, cannot be effectively treated, there remain a few where early recognition and treatment are of paramount importance. These include the development of paraplegia associated with extradural deposits of Hodgkin's tissue, acquired haemolytic anaemia, secondary infection including tuberculosis and the occurrence of peptic ulceration.

No dogmatic statement can be made as to the precise value of the treatment given to the patients in this series. The few untreated cases were largely selected in that the disease was advanced; similarly, the cytotoxic poisons were rarely used in either early cases or as the primary method of treatment. As regards irradiation, it does appear possible to eradicate local disease for a variable and, in some cases a considerable, length of time provided sufficient dosage is given. Heavy irradiation, in excess of 3000 r, is possible only when the disease is limited in extent, but it does appear a justifiable method of treatment in this type of case.

SUMMARY.

Two hundred and twenty seven cases of Hodgkin's disease are reviewed. The prognosis in an individual case appears to depend on many factors, including the stage of the disease at the time of presentation, the age of onset, the sex of the patient and the histological appearance. Progress of Hodgkin's disease is modified by treatment, but the true value of any form of therapy cannot be assessed unless the other prognostic factors are considered.

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THE THYMIC ORIGIN OF HODGKIN'S DISEASE.

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In this preliminary communication it is suggested that Hodgkin's disease is a tumour, and that it originates in the thymus.

Introduction.

In 1865 Sir Samuel Wilks gave the name " Hodgkin's Disease " to the clinical syndrome first described by Thomas Hodgkin in 1832. The histological features of this disease were added in the latter part of the last century when Greenfield (1878) noted the fibrous tissue and giant cells, Goldmann (1892) the eosinophils, and Sternberg (1898) the areas of necrosis with a detailed description of the giant cells. By 1902 the clinical, post-mortem and histological features of this disease were incorporated in the classical paper by Dorothy Reed. She concluded her article " We believe, then, from the descriptions in the literature and findings in eight cases examined, that Hodgkin's disease has a peculiar and typical histological picture, consisting of proliferations of the endothelial and reticular cells, formation of lymphoid cells, and characteristic giant cells, and a gradual increase of connective tissue, resulting in fibrosis and, in most of the specimens, in the presence of great numbers of eosinophiles."

Thus by the beginning of this century Hodgkin's disease was firmly established as a pathological entity. There followed a profusion of publications dealing with additional details of the various aspects of the disease, but in spite of the wealth of accumulated knowledge the precise nature of the disease remains obscure. This aetiological obscurity is best expressed by reference to the varied nomenclature in common use, which exposes our state of ignorance as to the real nature of the disease. Wallhauser (1933) listed an array of no less than 52 different names for Hodgkin's disease, and many more have been added since.

The more commonly used synonyms are lymphadenoma (Wunderlich, 1858) malignant lymphoma (Billroth, 1871) malignant granuloma (Benda, 1904) lymphogranuloma (Grosz, 1906) scirrhous lymphoblastoma (Mallory, 1914) fibromyeloid reticulosis (Pullinger, 1932) fibromyeloid medullary reticulosis (Robb-Smith, 1938). These names do at least indicate the aetiological opinions of their authors and focus attention on the views that Hodgkin's disease may be either a granuloma or a neoplasm.

Nature of the Disease.

Sternberg (1898) was convinced that tuberculosis was the cause of Hodgkin's disease, mainly because 8 of his 13 cases had a co-existent tuberculous infection.

He regarded the disease as " a peculiar type of tuberculosis of the lymphatic apparatus running the course of a pseudoleukaemia ". It was not until 1936 that he recanted from this dogmatic assertion.

Reed (1902) regarded the condition as a granuloma due to an unknown pathological agent and stated : " We are confident that if Hodgkin's disease exists in a gland the histology will give evidence of it, and that tuberculosis has no other relation to it than frequent association."

In spite of this clear statement to the contrary, Sternberg's tuberculous aetiology was followed by a series of publications, which neither proved nor disproved this view. Instead, during the bacterial studies on tissue affected by Hodgkin's disease, a large variety of other organisms were isolated and claimed by their founders to be the causative factor of the disease. None of these bacterial claims has been substantiated, and it seems probable that the various organisms were secondary invaders rather than the primary agents.

By 1924 Stewart and Dobson were able to summarize the aetiological possibilities as :

(1) An atypical form of tuberculosis.
(2) A specific infection by a diphtheroid bacillus.
(3) A granuloma of unknown aetiology.
(4) A neoplastic disease.

Twort (1930), after 6 years of extensive research into the aetiology of Hodgkin's disease by a wide range and variety of laboratory and clinical methods, was unable to shed any new light on the nature of the process, and concluded : " An assortment of the *in vivo* and *in vitro* experiments gave absolutely barren results, in fact, so invariably did the different experimental procedures we adopted lead to nothing, that one might have been dealing with a true new growth instead of what is generally accepted to be a granuloma."

Many contributors have postulated that Hodgkin's disease was a neoplasm and, therefore, cited the cell or tissue of origin instead of the aetiological agent.

Billroth (1871), Benda (1904), of the earlier contributors, alleged a neoplastic origin and called the disease malignant lymphoma and malignant granuloma.

Tsunoda (1911) is quoted as saying " the lymphoblast of the germinal centres is the offending element, the fibrosis and polymorphic histologic picture being a reaction to this stimulant, or to other secondary stimuli." Mallory (1914) also cited the lymphoblast and called the disease lymphoblastoma of the Hodgkin's type, or sclerosing lymphoblastoma.

Medlar (1931) saw a similarity in the giant cells of Hodgkin's disease to the megakaryoblasts, and placed the primary lesion of the disease in the bone marrow.

Piney (1926) classified Hodgkin's disease as a reticulo-endotheliosis. Pullinger (1932), adopting a similar view, alleged that the origin was from the mesenchymal reticulum cells and, therefore, the disease was an example of a reticulosis. Hodgkin's disease showed evidence of differentiation and was called a fibromyeloid reticulosis. Robb-Smith (1938) further elaborated this conception in his classification of tumours arising in lymph nodes and described Hodgkin's disease as a fibro-myeloid medullary reticulosis. This descriptive name has not gained universal acceptance, but the unitarian concept of the reticulum cell as the progenitor of *all* lymphoid tumours is the view most widely adopted as the origin of this group of tumours.

Ewing reiterated his previously published views that Hodgkin's disease was an infective granulomatous process, the causative organism of which was unknown though "tuberculosis follows Hodgkin's disease like a shadow" (Ewing, 1940, p. 416). He also states that Hodgkin's granuloma could transform into a sarcomatous process and that mediastinal Hodgkin's disease furnishes a large proportion of such cases.

Many examples of mediastinal Hodgkin's disease have been published, including those of Welch (1910) and Symmers (1911). Both these cases show definite evidence of invasion of the tissues, with metastases in one case to the skull and in the other to the liver in addition to the cervical nodes.

Ewing (1916) reviewed these cases of mediastinal Hodgkin's disease which were alleged to be sarcomatous and, on re-examining the sections of Symmers (1911) case, announced that this case was not Hodgkin's disease, but an example of a tumour originating in the thymus. Later Ewing (1940) says: "In a recent study of thymic tumours, I have collected evidence suggesting that many cases of Hodgkin's disease exhibiting sarcomatous qualities originate in the thymus, and that the peculiar characteristics of the infiltrating cells are referable to their origin from the epithelial reticulum cells of the thymus" (p. 415).

THE THYMIC ORIGIN OF HODGKIN'S DISEASE.

If Ewing's (1916) interpretation of Symmer's (1911) case is correct, it would appear that some tumours of the thymus must be taken into consideration when the histogenesis of Hodgkin's disease is discussed.

In 1900 Grandhomme used the term "thymoma" to describe all malignant tumours of the thymus regardless of their histological structure.

This grouping of all thymic tumours under one amorphous heading was unacceptable to many pathologists, including Ewing (1916), who subdivided thymomas as:

(1) Lymphosarcomas or thymomas to include lymphocytic and reticulum cell and giant cell tumours. The tumours simulating Hodgkin's granuloma were included in this group.
(2) Carcinoma.
(3) Spindle cell sarcoma or myxosarcoma.

It was in this paper that Ewing re-examined Symmers' (1911) case and negated the diagnosis of mediastinal Hodgkin's disease.

In spite of this, Symmers (1933) published his classification of thymic tumours thus:

(1) Perithelioma.
(2) Lymphosarcoma.
(3) Hodgkin's disease.
(4) Epithelioma.
(5) Spindle cell sarcoma.

Under the heading of Hodgkin's disease he described 5 cases in which the disease was localised to the anterior superior mediastinum. He was unable to differentiate the histological features of this tumour from the more widely disseminated disease, but did not mention that the generalised disease could have any relation to thymic tumour.

Decker (1935) adopted Ewing's (1916) method of classification and reviewed the literature of thymic tumours.

Andrus and Foot (1937) classified thymomas into two types, non-malignant and malignant. The malignant thymomas were divided into 7 sub-groups :

(1) Thymocyte or lymphocytoid type.
(2) Large celled or lymphoblast type.
(3) Thymic reticulum celled type.
(4) Perithelial type.
(5) Granulomatous type.
(6) Epithelial or carcinomatous type.
(7) Teratoid type.

They thus recognised the granulomatous type, which can mimic Hodgkin's disease, and state " there may be others in the literature reported in connection with Hodgkin's disease and therefore missed in our reviews. The picture is that of Hodgkin's granuloma located in the thymus or at its site."

Heuer and Andrus (1940) described a large series of mediastinal tumours of all types and included Hodgkin's disease of the thymus among the malignant thymomas, as did Wilson and Pritchard (1945).

These latter authors in their discussion of Hodgkin's disease in the thymus say :—

> " If the theory is correct that Hodgkin's disease is a reticulo-endothelial neoplasia, where is its histogenic source of origin in the thymus? As far as is known a reticulo-endothelial tissue capable of giving rise to lymphoid elements is lacking in the thymus. The epithelial reticulum might be considered as an analogous entity, but certainly not as identical in origin, function or characteristics. . . . There are two obvious alternatives to consider. One is that the more generally accepted theory regarding Hodgkin's disease is incorrect ; the other is that one is dealing with a reticular cell tumour of mixed cell nature attended by fibrosis."

It is suggested that both these alternatives are true if the words " reticular cell " are omitted from the last sentence.

In order to appreciate the extremely varied histological pattern of thymic tumours and their mode of spread, a knowledge of the normal development, the cellular characteristics and the lymphatic drainage of the thymus is essential.

Embryology and development of the human thymus.

The thymus gland of the human has a bilateral origin from part of each third pharyngeal pouch and is, therefore, endodermal in origin. A parathyroid gland also develops from the same pouch. The third pouch moves caudally with both the parathyroid and thymic rudiments. After the parathyroid has separated, the thymus has a thinner cranial and a broader caudal portion. The caudal part becomes incorporated in the upper part of the developing thoracic cavity, where it fuses with the thymic tissue of the opposite side. The upper cervical portion of the thymus usually disappears, but if it persists ectopic thymic tissue may result in the neck at the level of the thyroid gland (Boyd, 1950).

In the early stages the rudimentary thymus is composed of a closely packed mass of epithelial cells with vesicular nuclei and prominent mitoses (Fig. 2). After

the 30 mm. stage these epithelial cells become more loosely arranged and lymphocytes (of mesodermal origin) appear between the cells, probably by migration from the adjacent mesenchymal tissues. At the same time eosinophil polymorphonuclear leucocytes are present in the thymus gland (Fig. 8).

At 40 mm. the thymus becomes lobulated with fibrous trabeculae and now there is a medulla of paler staining epithelial cells and an outer darker staining cortex of lymphocytes. At about the 60 mm. stage the epithelial cells of the medulla begin to rearrange themselves into clumps to form the first stages of the Hassall's corpuscles. These stages are illustrated in Fig. 2–7 and were seen in a 140 mm. human foetus. The changes are firstly an increase in the size of the epithelial cells to give a large mononuclear cell with a vesicular nucleus and a prominent nucleolus (Fig. 2). The next stage is the " owl's eye " appearance with 2 of these same vesicular nuclei incorporated within the same cytoplasm to form a " mirror image " type of giant cell (Fig. 3 and 4). Further development results in nuclear aggregations containing a number of nuclei of identical appearance to the original cells (Fig. 5 and 6). Eventually the large fully formed Hassall's corpuscles are formed with their hyaline pink staining cytoplasm containing many nuclei (Fig. 7).

The important pathological aspects of this embryology are :

(1) There is a cervical portion of the developing thymus which may give rise to ectopic thymic tissues in the neck in 21 per cent (Gilmour, 1937) or in 20 per cent. of humans (Rieffel and Le Mée, 1909).

(2) A parathyroid, which develops from the same pharyngeal pouch, may have thymic tissue incorporated with it (Gilmour, 1937).

(3) The thymus has a mixed origin with endodermal epithelial and mesodermal lymphocytes.

(4) The developing Hassall's corpuscles, which arise from the epithelium are endodermal in origin.

(5) The Hassall's corpuscles in their development pass through the stages of a large mononuclear cell, a double nuclear " owl's eye " or " mirror image " type and subsequently a large giant cell form containing from 3 up to 20 or more nuclei.

(6) The thymus is divided into lobules by fibrous trabeculae.

(7) Lymphocytes are normally present in profusion in the thymus.

(8) Eosinophils are normally present in the thymus.

Lymph drainage of the thymus.

There is an efferent lymphatic ramification on each side that passes upwards and slightly posteriorly to drain into a lymph node on the jugular vein.

The anterior lymphatic vessels drain into the internal mammary group.

There are large lymphatic vessels on the posterior aspect of the thymus which drain into the tracheo-bronchial nodes (Rouvière, 1932).

Thus the lymph from the thymus can pass upwards into the neck on both sides, alternatively into the sternal and chest wall area and hence downwards in the internal mammary chain to the liver. It can also find a path into the tracheo-bronchial group, as a normal method of lymph drainage. It is also possible that ramifying lymphatics pass from this latter group into the thoracic duct.

Clinical material.

A series of 275 cases of Hodgkin's disease has been collected (some aspects of 227 cases of this series are reported in the preceding paper (Jelliffe and Thomson, 1955)). The larger series has been divided into three groups.

Group I.—Those patients with evidence of a mass in the thymic region at the time of presentation or within one year of diagnosis. There are 112 such cases.

Group II.—Those with evidence of mediastinal involvement at some stage of the disease, but not necessarily showing a definable mass in the thymic region. There are 120 such cases.

Group III.—Those patients with no evidence in the case notes of mediastinal involvement. There are 43 such cases, but the majority had been incompletely investigated.

Histological material is available from all these patients, and in every case the appearances are consistent with those classically described for Hodgkin's disease.

Table I summarises the clinical features of these groups of cases.

TABLE I.—*Clinical Features.*

	Group I. Thymic mass.	Group II. Mediastinal involvement.	Group III. No mediastinal involvement.
Number of cases	112	120	43
Average age	35	39	40
Sex	M. 51; F. 61	M. 86; F. 35	M. 27; F. 16
Average survival time from diagnosis	4 years, 2 months	3 years, 1 month	2 years, 7 months
Extent of spread:	%	%	%
Sternum	10	—	—
Chest wall	15	6	5
Lung	59	50	—
Bronchus	7	3	—
Oesophagus	3	1	—
Ribs	4	3	—
Heart	3	3	—
Scapula	2	2	—
Breast	4	—	—
Cervical nodes	88	86	80
Axillary nodes	50	65	60
Retroperitoneal tissues	43	72	36
Inguinal nodes	15	33	25
Spleen	32	55	40
Liver	18	28	26
Kidney	3	3	—
Pancreas	2	12	—
Stomach	1	2	—
Femur	1	4	5
Vertebrae	11	16	8
Humerus	1	—	—

The points of interest are that Group I has a lower average age than Groups II and III, a predominance of females and a better average prognosis.

From the point of view of spread it will be seen that, as would be expected, the sites most involved by the disease in Group I are the organs of the thorax. However 88 per cent showed invasion of the cervical nodes and 50 per cent the axillary nodes. The other commonly invaded sites are the upper abdominal lymph

nodes (43 per cent), the spleen (32 per cent) and the liver (18 per cent). There was evidence of bone involvement of the vertebrae in 11 per cent of the cases. A proportion also showed evidence of more widespread metastases to involve the humerus, the femur, and the groin (15 per cent).

In Group II the disease is more widespread, and although the thoracic area is inevitably involved, the axillary, retroperitoneal, splenic and hepatic sites show a higher incidence of involvement than the Group I cases. There is other evidence of further dissemination to involve vertebrae (16 per cent), pancreas (12 per cent), femur (4 per cent).

In none of the 43 cases of Group III did the case notes reveal any evidence of mediastinal disease. It will be noted that in this group there is no indication of involvement of any of the thoracic contents. This paradox is due to the fact that only some of these patients had a chest X-ray and many had no lateral radiograph. Many died within six months of presentation, and very few had post-mortem examinations.

Histological material.

The histological material can be divided into three groups according to the cellular appearances. These correspond to the Grade 1, 2 and 3 of the previous paper (Jelliffe and Thomson, 1955) and attention is drawn to the photomicrographs illustrating these three varieties of Hodgkin's disease.

The Hodgkin's disease, Grade 1, tumour is composed of a variable number of giant cells, usually of the " mirror image " or " owl's eye " type, buried in a mass of lymphocytes in which there are a few eosinophils also visible. This is identical in appearance to the histology of one type of mixed thymoma previously described by Lowenhaupt and Brown (1951). Fig. 9 shows the lobulated thymectomy specimen with the microscopy of this tumour illustrated in Fig. 13 and 14. The similarity of the Hodgkin's disease, Grade 1, and the thymic tumour is striking. The lymphocytes are abundant, the giant cells are comparable and the fibrous lobulation, noted by Harrison (1952) in his " benign Hodgkin's disease " are all represented.

The Hodgkin's disease, Grade 2, tumour is composed of mononuclear vesicular cells, " mirror image " giant cells and Dorothy Reed cells in a fibrous stroma, in which there are eosinophils, neutrophils and lymphocytes.

The appearances of this type of tumour are represented by Case 2, and the chest X-ray shows the thymic mass (Fig. 10). The histological picture of the cervical lymph node metastasis, which was removed at biopsy, is shown in Fig. 15 and 16. The appearances are those of accepted Hodgkin's disease.

The Hodgkin's disease, Grade 3, tumour is a more cellular, more obviously neoplastic example of the same pathological process. This tumour is represented by Case 3. The chest X-ray shows the thymic mass (Fig. 11). A cervical lymph node was removed for histological examination. This shows (Fig. 17 and 18) a cellular tumour composed of a background of actively proliferating mononuclear cells with visible mitotic figures and aggregations of these to form large giant cells. In addition, there are lymphocytes and polymorphonuclear leucocytes. The fibrous tissue, so prominent in the Grade 2 type of tumour, is sparse and areas of necrosis are also present.

There are, therefore, three recognisable microscopic patterns in Hodgkin's

disease, and each of them is associated with a tumour in the thymic region or in the mediastinum in 232 patients in the present series.

DISCUSSION.

The greatest obstacle to an understanding of the nature of Hodgkin's disease is the histological picture which appears to combine the manifestations of a granuloma and the behaviour of a neoplasm. The origin of the lymphocytes, the eosinophils and the fibrous tissue has been most readily explained on an inflammatory basis, while the giant cells have variously been regarded as atypical tuberculous giant cells, foreign body giant cells or tumour giant cells.

As we have already seen, all these cells are present in the normal foetal thymus, and it is suggested that the varied histological picture of Hodgkin's disease results from a tumour incorporating all the cells of the thymus gland. The resulting clinical manifestations of Hodgkin's disease are due to the spread and dissemination of this growth from the primary tumour in the thymus.

The rational classification of thymomata by Eisenberg and Sahyoun (1950) is based on embryological concepts and states that these tumours can arise from the epithelium, the lymphocytes or a mixture of both of these. The mixed tumour, therefore, consists of both epithelial and lymphocytic elements, and it is this type of thymoma that concerns us here. It is important to realise that either the epithelial or the lymphocytic elements may predominate in this group of mixed tumours, resulting in a diverse and variable histological picture, depending on which element predominates. Thus it is possible to picture a tumour at one end of this range consisting mostly of lymphocytes with few epithelial cells, a middle group consisting of an equal proportion of both epithelial and lymphocytic elements, and, at the opposite end, a tumour containing only scanty lymphocytes in a predominantly epithelial tumour.

In my view the Hodgkin's disease, Grade 1, is a mixed thymic tumour at the lymphocytic end of the scale. The thymic epithelium is represented by the giant cells buried among the lymphocytes and the fibrous stroma of the thymus by the fibrous lobulation of these tumours. The histology is identical to the "paragranuloma" of Jackson and Parker (1947) and "benign Hodgkin's disease" of Harrison (1952). The epithelial giant cells are also identical to the "owl's eyes" already seen in the developing thymus. I agree with Rosenthal (1936) and Lowenhaupt (1948) that the presence of a heavy lymphocytic infiltration in these tumours, being a normal developmental sequence in the thymus, indicates a well differentiated and, therefore, a slowly growing tumour.

I regard the Hodgkin's disease, Grade 2, as a mixed tumour of the thymus which is the pathological "half way house" of the mixed thymic tumour group. The lymphocytes of the thymus are always represented, but in varying degree. The epithelium of the thymus is present in the form of mononuclear cells, "mirror image" giant cells and Dorothy Reed giant cells all of which have their similarities to cells already seen in the developing thymus. These multinuclear cells may, in some examples, form larger cellular masses and approximate to the appearances of a Hassall's corpuscle (Fig. 26). When this occurs the tumour is readily acceptable as thymic in origin. This is also seen in Fig. 20 and 21 which represent an almost pure epithelial thymoma with profuse formation of Hassall's corpuscles. The adjacent epithelial cells of this tumour can be seen to be very similar to

many of the cells seen in Hodgkin's disease, although this tumour is clearly of thymic origin and the histological picture, because of the epithelial differentiation and the lack of the other elements, does not simulate the pattern of Hodgkin's disease.

Eosinophil polymorphonuclear leucocytes are a common constituent of the normal thymus, and in Hodgkin's disease these cells are usually present in profusion. Whether they are merely attracted by the presence of additional bulk of thymic epithelium or whether they are an integral part of the tumour remains a matter for conjecture.

The fibrous tissue, which often forms a large part of the neoplastic process, is either a reaction on the part of the tissues to the tumour cells, or is the neoplastic representation of the fibrous lobulation of the normal thymus gland.

Hodgkin's disease, Grade 2, is, therefore, a neoplastic mixture incorporating all the elements of thymic tissue buried in a fibrous stroma of variable density, quantity and cellularity.

The Hodgkin's disease, Grade 3, is a more cellular tumour at the epithelial end of the mixed thymoma scale. The mononuclear tumour cells are epithelial in origin and the giant cells represent abortive and bizarre Hassall's corpuscles. The appearances of these epithelial elements again have their similarities in the developing thymus. The tumour cells evoke less fibrosis, and both the lymphocytes and eosinophils are less numerous. The areas of necrosis are an expression of a rapidly growing tumour, whereby the blood supply is insufficient for the needs of the tumour tissue.

Eisenberg and Sahyoun (1950) describe 7 cases of mixed thymic tumours, all of which had previously been diagnosed as Hodgkin's disease. Only one of these cases was suspected of having a mediastinal primary neoplasm on clinical grounds alone. Chest X-rays showed widening of the superior mediastinum in 5 cases, but the other 2 were radiologically normal. Six of the patients subsequently died following treatment. The extent of spread in these patients shows a remarkable similarity to the 112 cases of Hodgkin's disease already reported in Group I. All had cervical lymph node involvement and a mass in the superior mediastinum. Three showed evidence of pulmonary infiltration, 4 had an enlarged spleen and 2 developed an enlarged liver. Two cases had multiple vertebral deposits and in all there was widespread lymph node invasion. An average prognosis of 53·2 months' survival following the onset of symptoms compared with an average of 37·2 months for cases of Hodgkin's disease previously reported by the same authors (Sahyoun and Eisenberg, 1949).

The interest of Eisenberg and Sahyoun's (1950) publication centres around the striking similarity of the clinical manifestations, the sites of spread and the histological appearances of these thymic cases to those of Hodgkin's disease, in spite of the descriptive and photomicrographic appeals to the contrary.

Lowenhaupt and Brown (1951) published a series of 9 cases of the granulomatous type of thymoma and extracted other examples, considered to represent this type of tumour, from the literature.

At the time of diagnosis all 9 cases had a superior mediastinal mass and 6 had either cervical or axillary node enlargement or chest wall invasion.

Five of the 9 patients subsequently died and the details of the extent of spread of the tumour found at autopsy are described. All showed mediastinal involvement with invasion of either the hilar nodes or the lung. There was cervical

lymph node invasion in 5 cases, axillary lymph node and chest wall invasion in 3 cases, with liver invasion in 1 case and splenic involvement in another. The diaphragm was involved in 1 case and the retroperitoneal tissues in 2 cases where the tumour had extended to surround the adrenals, bile ducts, pancreas and posterior aspect of the stomach. Four patients remain alive from $1\frac{1}{2}$ to 5 years later, but 3 have a residual mediastinal mass.

The surviving patients of their series, therefore, have the disease localised to the thymic region with evidence of invasion of the adjacent mediastinal tissues, the cervical or axillary lymph nodes. The fatal cases showed mediastinal, pulmonary and abdominal invasion with either retroperitoneal, splenic or liver involvement. In these fatal cases the extent of spread is therefore analogous to many examples of disseminated Hodgkin's disease and is comparable to the cases in Group I of the present series.

Lowenhaupt and Brown's (1951) paper, with excellent reproductions of chest X-rays, operation specimens and photomicrographs of the histology, deals not only with the extent but also with the method of spread. The authors describe the routes of extension of tumour from the thymic region as anteriorly to involve the sternum, superiorly to the cervical lymph nodes and posteriorly to the tracheobronchial group at the hilum of the lung. These routes of spread follow, therefore, the normal lymphatic paths. There are, in addition, other possible modes of spread from the internal mammary chain to the liver and in the ramifications from the hilar lymph nodes to the thoracic duct to involve the retroperitoneal tissues by retrograde spread.

Both Eisenberg and Sahyoun (1950) and Lowenhaupt and Brown (1951) describe differences between the reticulo-endothelial cells of Hodgkin's disease and the reticulo-epithelial cells of thymic origin. They also note that the thymic giant cells are two to four times larger than the Reed-Sternberg cells of Hodgkin's disease.

In spite of the list of reasons why the mixed cell or granulomatous type of thymoma differs from Hodgkin's disease, I am reluctant to assume that they are, in fact, two separate diseases. The age groups and the clinical manifestations are similar and the sites of dissemination appear identical. A more rational approach is to regard the mixed thymomata and Hodgkin's disease as the same disease process and the variable histological appearances encountered as dependent on the balance between the epithelium and the lymphocytes in an individual tumour, and also on the degree of differentiation of the epithelial cells. With epithelial differentiation more definitive Hassall's corpuscles will be formed and, therefore, the giant cells will be larger. This accounts both for Haagensen's (1932) statement that the giant cells of mediastinal Hodgkin's disease "are different from those of Hodgkin's granuloma," and for the observations of Eisenberg and Sahyoun who describe the thymic giant cells as being larger than those of Hodgkin's disease.

In the present series of 275 cases there were 43 patients with no evidence in their case notes of mediastinal involvement.

Can, therefore, a thymic origin be advanced for all cases of Hodgkin's disease? This is difficult to answer, but a tumour in the thymic region, if small, is notoriously difficult to diagnose. As Jackson and Parker (1947) stress, there is "the necessity for roentgen-ray study of the chest when the patient is first seen, even though there are no symptoms or signs even remotely suggesting such lesions." A lateral chest

X-ray is also essential, as this is the sole method of localising a small tumour in the superior mediastinum, which does not encroach beyond the lateral margins of the sternum (Blalock, 1941). In Group III, many of the patients, having no thoracic symptoms had no chest X-ray and of those that did, many had no lateral radiograph. This certainly is one reason why mediastinal involvement was not detected.

Another possible reason is that this group has a worse average prognosis than Groups I and II, and 16 patients died within a year of diagnosis. It is probable that in these cases the thymic tumour, at the time of death, was not sufficiently large to be detectable clinically. Some of the Group III cases of the present series may, therefore, be similar to many examples of bronchial, mammary and nasopharyngeal tumours which often develop widespread metastases from an occult primary neoplasm.

A further explanation for failure in any type of case to recognise the primary enlargement of the thymic tumour, is that the thymoma may undergo sclerosis due to the fibrous tissue of the tumour. This may occur as part of the natural history of the disease, even before irradiation therapy. Fig. 12 shows a small tumour removed from the thymic region in a case of Hodgkin's disease before any irradiation had been directed at the mediastinum. The illustration shows the prominent fibrosis and the tumour cells are limited to small foci in the dense collagen.

As previously stated, ectopic thymic tissue is present in the neck of a significant proportion of human beings. It is not uncommon to find histologically recognisable thymic tissue removed during block dissections or other surgical procedures in the neck region (Fig. 19).

Gilmour (1937) found ectopic thymic tissue in 21 per cent of dissections of the neck region and Rieffel and Le Mée (1909) in 20 per cent. It is conceivable that tumours of the mixed type may arise from this ectopic thymic tissue and account for the lack of a mediastinal tumour, the primary neoplasm being in the neck.

CONCLUSIONS.

In my view the embryology of the developing thymus gland gives the clue to the histogenesis of Hodgkin's disease. The tumours of the thymus can be either purely epithelial, purely lymphocytic or a mixture of these. The mixed tumour group is composed, therefore, of both epithelial and lymphocytic elements, and forms the basis of Hodgkin's disease. The mixed tumours show evidence of differentiation along two lines. If there is a profusion of lymphocytes and little epithelium the appearances are those of a Hodgkin's disease, Grade 1. If the epithelium is fully differentiated Hassall's corpuscles are formed in profusion and the tumour is then epithelial, thus outside the range of histological confusion with Hodgkin's disease. A less differentiated epithelial tumour with an admixture of lymphocytes shows both mononuclear cells, giant cells, lymphocytes, eosinophils and fibrous tissue. This is histologically indistinguishable from Hodgkin's disease, Grade 2, the ordinarily accepted picture of Hodgkin's granuloma.

Hodgkin's disease, Grade 3, has an epithelial preponderance, and is a less differentiated example of this mixed tumour with less fibrosis, fewer lymphocytes and eosinophil polymorphonuclear leucocytes. In all these mixed tumours the

epithelial cells of both mononuclear and giant cell types have their histological counterparts in the developing foetal thymus.

The failure to find mediastinal involvement in cases of Hodgkin's disease is due to a variety of reasons, but the three probable causes are lack of a chest X-ray, a tumour originating in an ectopic thymus in the neck, or a sclerosis of the primary due to fibrosis.

EXPLANATION OF PLATES.

FIG. 1.—Foetal thymus. Section shows a cross-section of a foetal thymus with a cortex and medulla at 16 weeks. × 12.

FIG. 2.—Foetal thymus. Showing mononuclear vesicular cells in the medulla of the thymus. Note the mitoses. × 800.

FIG. 3.—Foetal thymus. Showing a " mirror image " giant cell in the developing thymus. × 800.

FIG. 4.—Foetal thymus. Showing a " mirror image " giant cell with increased prominence of the nucleoli to give the " owl's eye " appearance. × 800.

FIG. 5.—Foetal thymus. Showing a giant cell with four nuclei centrally arranged. × 800.

FIG. 6.—Foetal thymus. Showing a large multinucleate giant cell. × 800.

FIG. 7.—Foetal thymus. Showing a Hassall's corpuscle. × 800.

FIG. 8.—Foetal thymus. Showing eosinophil polymorphonuclear leucocytes in the developing thymus. × 800.

FIG. 9.—Case 1. Showing the thymectomy specimen. The fibrous lobulation is visible. The longest axis measured 8 cm.

FIG. 10.—Case 2. The chest X-ray shows the thymic mass.

FIG. 11.—Case 3. The chest X-ray shows the thymic mass.

FIG. 12.—Showing a fibrotic mass containing small foci of tumour removed from the thymic site before irradiation. × 1.

FIG. 13.—Case 1. Showing giant cells among lymphocytes. × 100.

FIG. 14.—Case 1. Showing the giant cells surrounded by lymphocytes. This is the histological pattern of Hodgkin's disease, Grade 1. × 500.

FIG 15.—Case 2. Showing a pleomorphic tumour with giant cells and bands of fibrous tissue. × 100.

FIG. 16.—Case 2. Showing the histological picture of Hodgkin's disease, Grade 2, with giant cells, lymphocytes and polymorphonuclear leucocytes. × 500.

FIG. 17.—Case 3. Showing a cellular pleomorphic tumour with conspicuous giant cells. × 100.

FIG. 18.—Case 3. Showing the pleomorphism of the tumour with an " owls' eye " type of giant cell, densely staining giant cells, polymorphonuclear leucocytes and some fibrous tissue. This is the histological pattern of Hodgkin's disease, Grade 3. × 600.

FIG. 19.—Showing ectopic thymus tissue removed from the neck during a thyroidectomy. × 100.

FIG. 20.—Epithelial thymoma. Showing an epithelial thymoma with recognisable but atypical Hassall's corpuscles. × 500.

FIG. 21.—Epithelial thymoma. Showing an epithelial thymoma with a Hassall's corpuscle and mononuclear tumour cells. × 500.

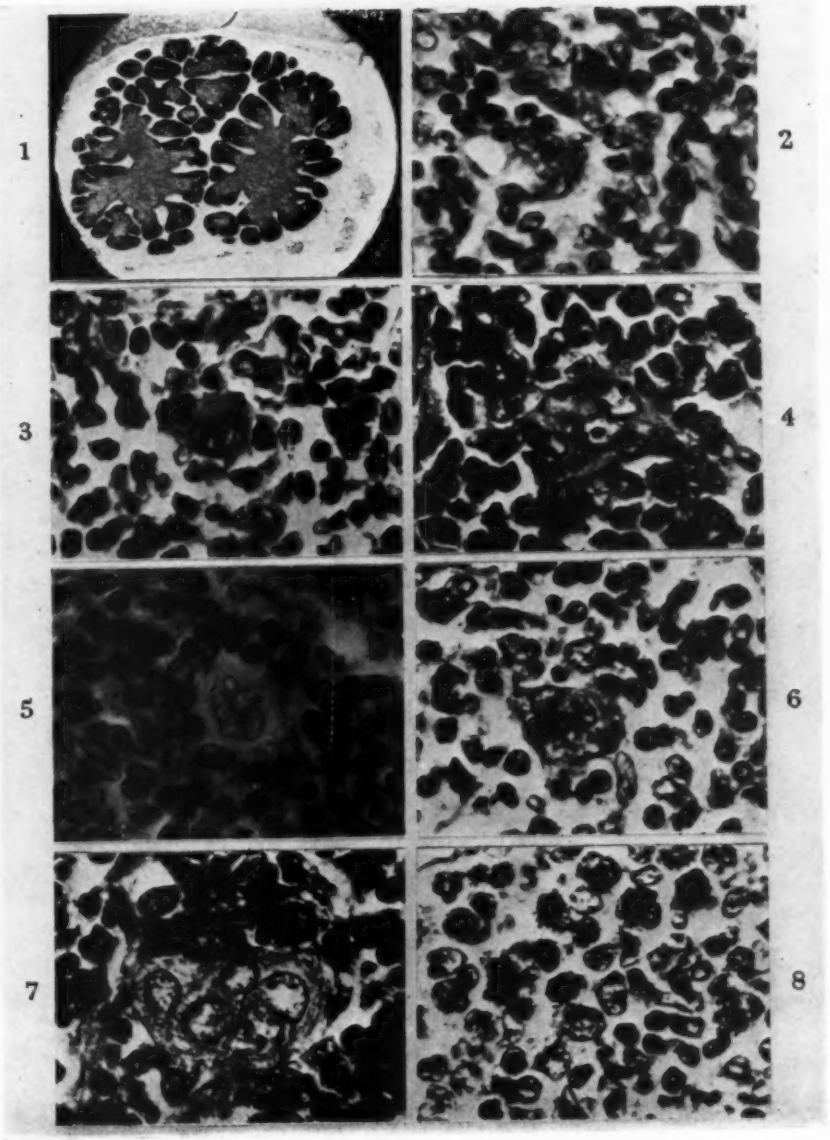
FIG. 22.—Showing " owl's eye " giant cells in a lymphocytic stroma. × 500.

FIG. 23.—Showing " owl's eye " giant cells among lymphocytes and polymorphonuclear leucocytes. × 500.

FIG. 24.—Showing a multinuclear giant cell in a fibrous stroma with lymphocytes and polymorphonuclear leucocytes. × 500.

FIG. 25.—Showing giant cells with prominent nucleoli surrounded by lymphocytes. × 500.

FIG. 26.—Showing a Hassall's corpuscle among lymphocytes. × 500.



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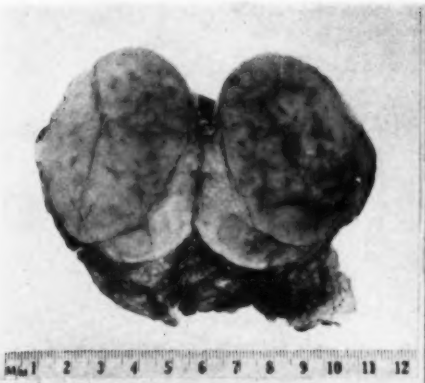

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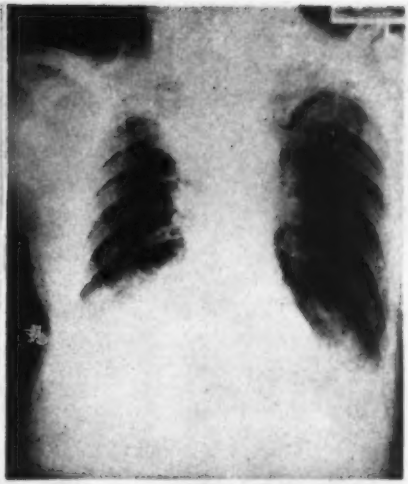

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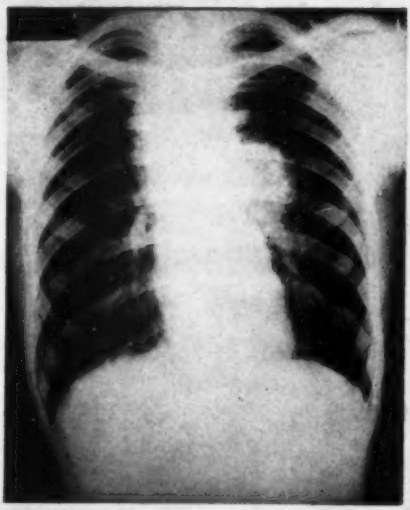

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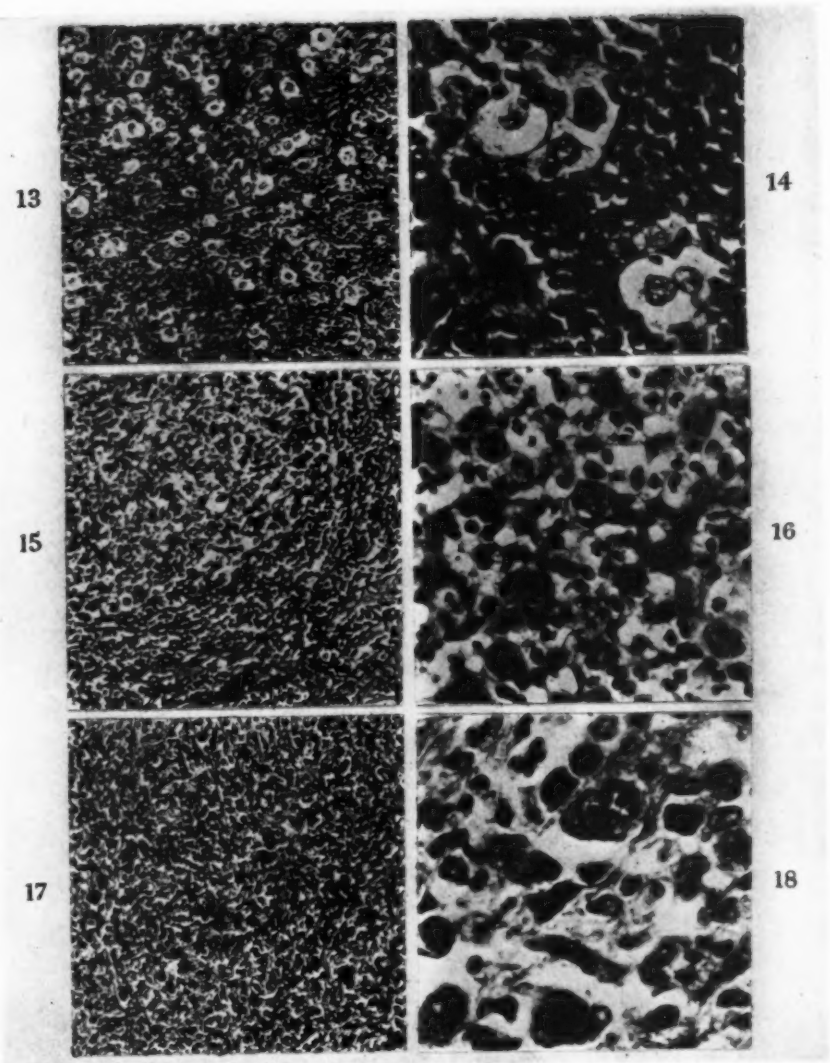

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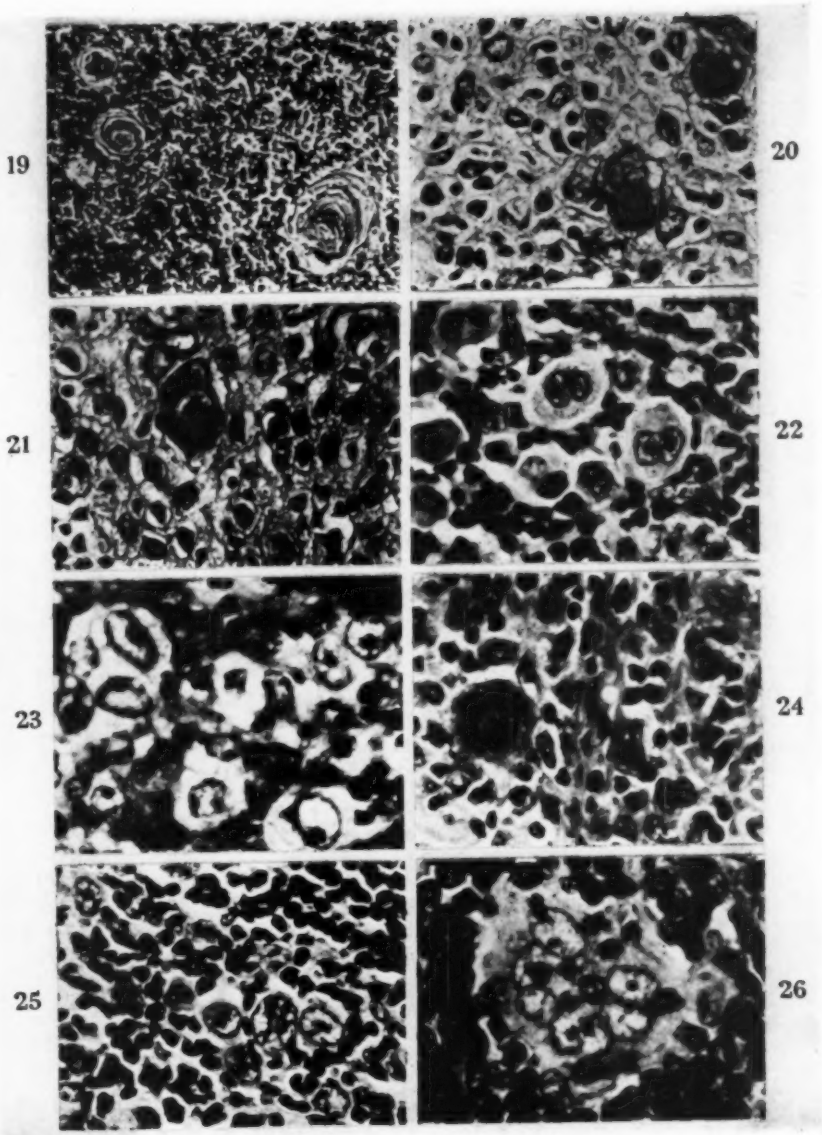

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The spread of these mixed thymic tumours can be accounted for by a study of the normal lymphatic drainage of the thymus, whereby lymphatic routes extend to the neck, to the chest wall, to the hilar and retroperitoneal lymph nodes.

The reason for the longer survival of patients with thymic tumours restricted to the mediastinum is that they are histologically better differentiated. The well-differentiated epithelial elements lead to a diagnosis of a thymoma of the Hodgkin's type, while if there is even better differentiation, with the formation of diagnostic Hassall's corpuscles, the observer will then call the tumour in question an epithelial thymoma.

In conclusion, therefore, all the essential cellular features of Hodgkin's disease are normally present in the developing thymus. Tumours incorporating a mixture of all these elements, the epithelium with its giant cells, the lymphocytes, the eosinophils and the fibrous tissue—produce the clinical, the histological and the post-mortem features of a syndrome known for over a hundred years as Hodgkin's disease.

SUMMARY.

It is postulated that Hodgkin's disease has its origin in the thymus gland.

The thymus is composed of epithelial cells, from which are derived the multinucleate Hassall's corpuscles ; there are also lymphocytes, eosinophils and fibrous tissue present.

Hodgkin's disease is regarded as a tumour of the thymus gland in which all these cellular elements, are incorporated in varying degrees to give the histological appearances of Hodgkin's disease.

Histological and pathological evidence, from a series of 275 cases of Hodgkin's disease, is put forward in support of this concept.

The tumours reproduce the appearance of many of the cells seen in the normal developing thymus and an appreciation of the normal lymphatic drainage of the thymus accounts for the sites of spread of Hodgkin's disease.

Where no mediastinal involvement is demonstrable some of the possible reasons are discussed.

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INTRACRANIAL HETEROTRANSPLANTATION OF HUMAN TUMOURS.

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NUMEROUS attempts have been made to propagate human tumours in laboratory animals.

Woglom (1913) in a review on the study of experimental cancer, stated: "Since the day when Peyrilhe made the first recorded experiment, attempts to transfer cancer from man to the lower animals have been almost continuously in progress, and although a positive result has been reported more than once the consensus of opinion has been for many years that such claims cannot be seriously entertained."

Woglom relates how Peyrilhe in 1775 procured about two drachms of cancerous virus from a cancerous breast and introduced it into a small wound made in the back of a dog. His maid, however, was "disgusted by the stench of the ulcer and, softened by the cries of the animal, terminated the experiment by killing the dog".

Some of the early attempts at intracerebral inoculation of human cancer were reviewed by Sailer (1900). He quotes Geissler (1891), who inoculated human carcinoma into the brains of 25 rabbits and in those that were killed for study, the fragments of carcinoma, although tolerably well preserved, exhibited no tendency to proliferate, and produced exceedingly little reaction in the surrounding nervous tissue.

Sailer also refers to Trasbot who for "thirty years performed numerous experiments, injecting carcinomatous substances and material into animals, with only one successful tumour, which developed in the course of a few weeks at the site of inoculation and then gradually atrophied".

In concluding his critical review Sailer said that "the transmission of tumours to the lower animals from human beings may be regarded as absolutely impossible, unless some profound modification in technique or in the preparation of the animals subjected to the experiments shall be devised".

Ehrlich (1907), quoted by Selbie (1936) demonstrated that a mouse tumour could grow progressively in the rat for a period of 8 to 10 days, after which it invariably regressed. By zig-zag transplantation between rat and mouse, Ehrlich was able to cultivate a mouse tumour through fourteen passages.

Between 1913 and 1930 reports of the successful heterologous transplantation of animal tumours were published by Murphy (1913), Shirai (1921), Putnoky (1930) and others.

Selbie (1936), after transplanting the Ehrlich-Putnoky mouse tumour subcutaneously into rats, made detailed histological studies of the temporary

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survival and proliferation of the tumours, and concluded that the transplanted animals could in no sense be said to be suffering from cancer and that heterologous transplantation was of little importance in the study of cancer.

Greene (1938), reported the slow but progressive growth of a human scirrhous carcinoma of the breast for more than 80 days in the anterior chamber of the eyes of 7 out of 12 rabbits used. Growth was first apparent towards the end of the third week. Other papers by Greene (1941, 1942, 1946, 1948, 1949, 1951, 1952, 1953, 1954) described success in transferring animal and human tumours to the anterior chamber of the eye and brain of alien species. Adult normal tissues, benign tumours, hyperplasias, so-called pre-cancerous lesions and inflammatory granlomas were incapable of growth after heterologous transfer, but some embryonic tissues and cancers survived and grew.

No morphological criteria could be found to distinguish transplantable and non-transplantable tumours. Histologically the transplants were usually identical with the parent tumour.

Greene (1952) reviewed his results between February, 1939, and February, 1950, consisting of 127 transplantation experiments from 123 different patients, and suggested that heterotransplantability differentiates phases of tumour development rather than varieties of tumour, and that non-transplantable tumours represent early developmental phases, and the transplantable tumours a later stage.

Transplants were performed by Greene himself into one eye of 6 to 8 guinea-pigs for each experiment. The following table, taken from his paper, shows the relationship between the state of the patient at the time of transfer and the results of transplantation (Table I).

TABLE I.—*Relationship between State of Patient and Results of Transplantation (Greene).*

	No involved nodes or metastases.	Local nodes involved.	Distant nodes involved.	Organ metastases.
Number of cases	45	41	9	28
Number of cases successfully transplanted	13	16	8	28
Percentage of cases successfully transplanted	28·8	39·0	88·8	100

Other workers have been less successful in their attempts.

Morris, McDonald and Mann (1950) made transplants of 40 malignant tumours of human beings into the anterior chambers of 167 guinea-pigs, producing only 1 successful implant, and suggested that successful heterologous transplantation of tumour tissue as a biological test of malignant disease was not a feasible procedure.

In 1951 Andrus, Meissner and Whorton transplanted 38 human malignant tumours and 10 human lymphomas into the anterior chambers of the eyes of 383 guinea-pigs. Generally six to ten animals were used for each tumour. Twelve transplants from 6 of the tumours showed unequivocal evidence of growth (3 melanocarcinomas, 2 bronchogenic carcinomas and 1 renal-cell carcinoma). In 4 of these cases metastatic tumour was used for transplantation. None of the 10

lymphoma transplants showed evidence of growth. They concluded that in their hands the low incidence of positive anterior chamber transplants definitely limits the value of the procedure as a diagnostic test.

Greene (1951) reported the successful growth of human cancer in the guinea-pig and mouse brain; the majority of the mice died between the 70th and 90th days. He concluded that the rapidity with which human cancer grew in the mouse brain together with the low cost of mice were factors of importance in the clinical use of heterotransplantability as a prognostic test. It was found that with the exception of brain neoplasms, "only metastasizible tumours were heterotransplantable, and this fact was utilized to determine the status of human tumours with respect to metastasizability at the time of their removal".

It was suggested that the mouse brain could be used in place of the guinea-pig's eye with advantage from the standpoint of both time and economy.

Eichwald, Goodman and Chang (1951) found that tumours of mouse sarcoma 37 grew to a larger size without regression in the sub-dural space of guinea pigs than in similar transplants to the guinea-pig eye.

Green and Whiteley (1952) showed that cortisone strongly depressed antibody production to iso-antigens, and heterologous antigens in transplanted tumour tissue. They described the absence of cellular reaction at 6 to 16 days around human "oat-cell" carcinoma transplanted subcutaneously in 5 mice receiving cortisone. The implants showed evidence of non-invasive tumour growth at the periphery. There was a very considerable reaction around the implant in the control mice. Toolan (1953) reported success in transplanting human tumours subcutaneously into cortisone-treated X-irradiated weanling rats.

This investigation was undertaken to confirm the fact that human tumours could be grown within the skull of the mouse, and to explore the possibilities of this method as a biological test for malignancy. It was hoped to increase the number of "takes" by conditioning the host with cortisone.

This paper is a report on the results of the intracranial implantation into 180 mice of preparations of 20 human malignant tumours from 19 patients.

METHODS AND MATERIAL.

White mice of both sexes, inbred but not of a pure strain, between 30 and 161 days old at the time of implantation were used. They were fed on a standard cube diet. Intraperitoneal avertin (approximately 0·1 ml. of a 2·5 per cent solution per 10 g. body weight) was used as an anaesthetic. This gave satisfactory deep anaesthesia for 20 minutes or longer, and the animals were sleepy for some hours after the operation.

Under anaesthesia the skin over the head was shaved with a scalpel blade. No antiseptic was put on the skin, but sterile instruments were used.

Three methods for introducing the tumour material into the brain were used, depending on the nature of the material available.

(1) A right paramedian saggital incision down to the bone was made and the skull then drilled with a modified dental drill 1½ mm. in diameter and projecting about 2 mm. from inside a small metal cylinder which acted as a guard when the skull had been penetrated.

This method was used when small pieces of solid tumour about 1½ mm. in size were directly implanted into the brain after puncturing the dura. The tissues

were pushed into the brain with a fine pair of forceps or from a modified Bashford needle used for implanting tissue as described below.

(2) In some instances scrapings from the cut surface of the tumour were drawn into a modified Bashford needle consisting of a closely-fitting stilette projecting 2 mm. beyond a short-bevelled needle mounted on a handle. In tumours of suitable consistency it was possible to use the stilette as a plunger and suck material directly into the needle. The needle was then inserted into the brain through the intact skull, the stilette was depressed and the tumour material deposited intracranially. The skin incision was closed with one small Michel clip.

(3) If the tumour was sufficiently fluid (e.g. in centrifuged suspensions of pleural fluid or bone marrow) the material was drawn into a Mantoux syringe and approximately 0·025 ml. inoculated through a No. 16 or 18 guage needle.

Some of the mice received one post-operative injection of a suspension containing 3000 units of procaine with penicillin G and 1000 units of sodium penicillin G. 1·25 mg. cortisone acetate was given subcutaneously daily to some of the mice 1 to 3 days before operation and continued daily for the first few days. Subsequently the dose was reduced as signs of intolerance developed.

Bacteriological culture was not performed on the implanted tissue, but intracerebral abscesses were only occasionally found.

The material used was received from the operating theatres of the Middlesex Hospital, the Soho Hospital for Women and the Harefield Hospital for Diseases of the Chest. Autopsy material was obtained from the Bland Sutton Institute of Pathology. Portions of tissue adjacent to those implanted were sectioned, or smears made from scrapings and centrifuged deposits.

Material was implanted into the mice within 3 hours of receipt from the theatre.

Naturally during the operative procedures in removing various tissues from the patient some of the cells may have been deprived of their blood supply for longer periods. In the case of post-mortem material delay was up to 12 hours from death.

Animals that had not died were killed at various times up to 300 days after implantation. As soon as possible after death the skin over the dorsum of the skull was removed and the skull and brain cut through with a sharp scalpel in the region of the burr-hole, or the fracture made by the Bashford needle. The heads were fixed in formal-saline, decalcified, and sections cut in the region of the implanted area. In cases where intracerebral inoculation was performed with a Mantoux syringe, sections of the brain were made in the region of the inoculated area.

DEFINITION OF A " TAKE ".

When interpreting the results of intracranial transplantation it is essential to define what is meant by a " take " or a " positive result ".

The mere survival of cells which appear viable using standard histological criteria must be distinguished from growth where, as a result of reproduction, there is an increase in the total number of transplanted tumour cells with a progressive infiltrating or expanding tumour showing numerous mitoses.

Lumb (1954*a*, 1954*b*) reporting on the results of the transplantation of 60 human tumours into mouse brains, made serial sections to demonstrate the histo-

logical appearances of the implanted tissue up to 28 days and in 3 cases up to 35 days. Isolated clumps of cells sharply demarcated from adjacent brain and appearing " viable " using normal methods of histology were seen in 40 cases. He stated : " It is obvious that if a transplanted tumour grew as a solid mass to destroy a considerable area of host tissue then no doubt would remain that frank growth was taking place in the transplanted area." He found no such growth and only in 2 cases was there evidence of infiltration by tumour cells at the edge of the inoculation area.

It is evident that even after 35 days the presence of apparently viable cells is no criteria of growth ; local invasion is very suggestive, but it seems likely that only increase in size due to an increase in the total number of tumour cells is absolute proof of growth.

Kemler and Graham (1950) studying the influence of sex hormones on successful heterologous transplants of human bronchogenic carcinoma in the anterior chamber of guinea-pig eyes defined a " take " as at least a twofold enlargement of the transplant with definite vascularization. They used fragments of tumour tissue, 1 to 2 mm. in size and obtained growth in 6 of 29 attempted transfers in female guinea-pigs and 4 out of 29 in males.

Towbin (1951) evaluated the results of 100 different malignant human tumours transplanted into the anterior chamber of the guinea-pig's eye and described three phases in the tumour development.

(1) *Suspension stage*, in which the transplant exists in a "tissue culture " state, deriving nourishment from the surrounding medium.

(2) *Nidation stage*, during which the transplant becomes attached and vascularized.

(3) *Growth stage*, during which active proliferation of the tumour elements is manifested.

Of the 100 different malignant human tumours studied by Towbin, 89 tumours showed early regression of transplanted tissue ; 9 tumours persisted in the stage of nidation for 10 to 114 days, and only 2 tumours (a fibrosarcoma and a carcinoma of the maxillary sinus) showed active growth after long latent periods in the anterior chamber.

The results indicated that relatively few human tumours, though clinically malignant, could be transplanted successfully to the anterior chamber of the guinea-pig's eye and that this factor precluded the use of the technique as a practical measure for differentiating benign from malignant tumours.

RESULTS.

Of the 20 human tumours in this series only 3 (from Patients F, J and C with lung cancer) showed evidence of infiltrative or expansive growth in a few of the mice implanted. In these instances, which have been reported briefly previously (Chesterman, 1953), the type of growth within the mouse skull was classified as follows :

Macorscopic tumours : Two tumours (Cases F and J) were placed in this group (Fig. 3, 4, 5, 6, 8). They formed expanding growths after a period of 60 days in the host, easily visible to the naked eye on section of the skull, and about half the diameter of the mouse's brain when seen on cross section. They grew as a solid mass compressing the host tissue. On histological examination they

were vascularized, organized and showed numerous mitoses. In general, they were sharply demarcated from the surrounding brain, but in places showed infiltration.

Microscopic tumours: This term was applied to transplants of over 12 days' duration, not clearly seen by the naked eye, but showing on section unequivocal evidence of invasion of the host tissue or, as seen in one mouse, of the stroma surrounding malignant cells in the implant. Four mice were placed in this group, two in Case F, one in Case J, and one in Case C.

The presence of cells appearing "viable" on the usual histological criteria was found in the brains of two cortisone-treated mice dying at 7 and 10 days bearing implants from two human malignant melanomata. Six and 12 mice were originally implanted.

"Viable" cells were also seen in one cortisone-treated mouse dying at 7 days and bearing an implant from a mixed squamous and adenocarcinoma arising in an ovarian dermoid cyst (15 mice originally implanted) (Fig. 11 to 15).

EXPLANATION OF PLATES.

FIG. 1.—Coronal section of head of normal mouse. × 3¼.

FIG. 2.—Histological preparation of a coronal section of a normal mouse's head. H. & E. × 3¼.

FIG. 3.—Case F. Coronal section of head of mouse showing a "macroscopic tumour" in the middle of the brain at 128 days. × 5.

FIG. 4.—Case F. Low power microphotograph of "macroscopic tumour" in the brain at 128 days. H. & E. × 7.

FIG. 5.—Case J. "Macroscopic tumour" at 66 days showing vascularized tumour expanding overlying skull. H. & E. × 8.

FIG. 6.—Case J. High power of tumour shown in Fig. 5. H. & E. × 640.

FIG. 7.—Case J. Section of original subcutaneous deposit of secondary carcinoma used for transplantation. H. & E. × 640.

FIG. 8.—Case J. Coronal section of head of mouse showing appearance of "macroscopic tumour" at 66 days. Largest diameter of tumour 5·5 mm.

FIG. 9.—Case F. Section of edge of tumour at 128 days showing blood vessel. H. & E. × 320.

FIG. 10.—Case C. Microscopic growth at 56 days in the brain of a cortisone-treated mouse adjacent to a portion of "viable" bone pushed into the brain during the operation. H. & E. × 340.

FIG. 11, 12 and 14.—Sections showing areas of squamous cell carcinoma and adenocarcinoma in the original malignant ovarian dermoid cyst. H. & E. × 320.

FIG. 13 and 15 show a few isolated clumps of cells and some stroma in the brain of a cortisone-treated mouse dying 7 days after implanatation with material from the ovarian tumour shown in Fig. 11, 12 and 14. H. & E. × 320.

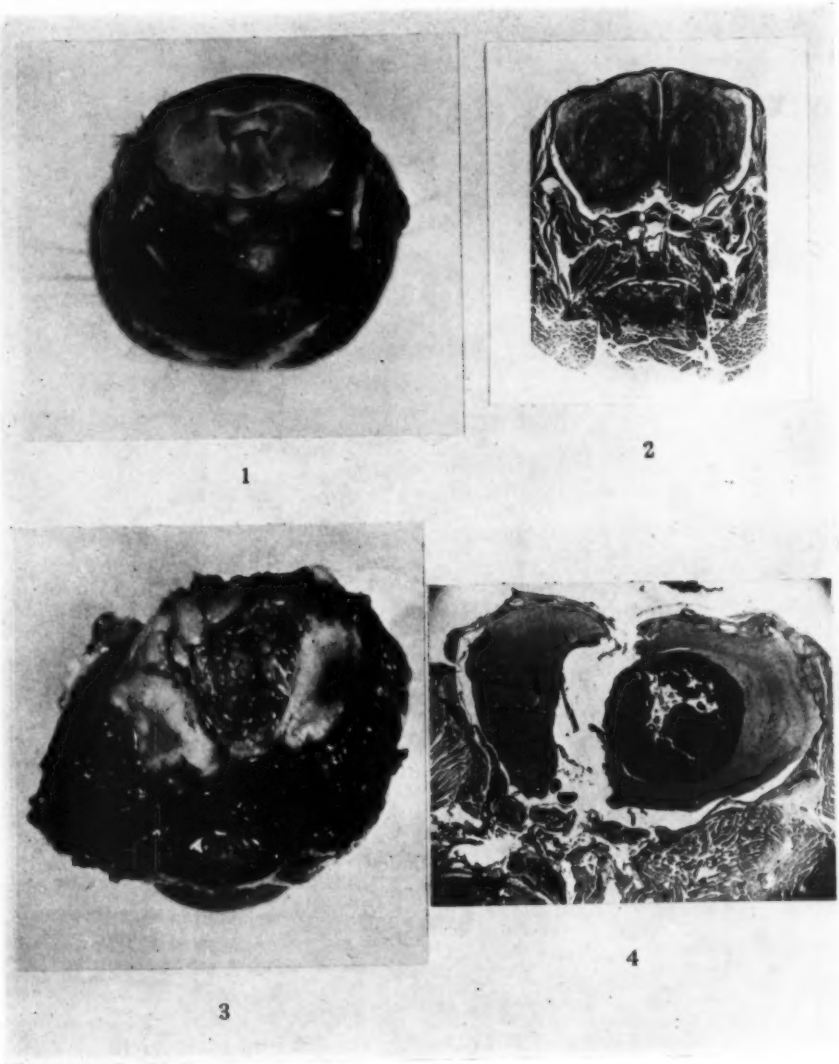
FIG. 16.—Case J. Vascularized tumour 43 days after implantation growing in ventricle and adjacent brain (cortisone-treated mouse). H. & E. × 35.

FIG. 17.—High power of Fig. 16 showing growth replacing ependymal lining and invading brain. Numerous mitoses. H. & E. × 185.

FIG. 18.—Case F. "Microscopic growth" at 13 days in lateral ventricle and choroid plexus from intracerebral inoculation of scrapings from the cut surface of the tumour. H. & E. × 35.

FIG. 19.—Case F. High power of Fig. 18, showing invasion of the brain substance. H. & E. × 185.

FIG. 20.—Implanted tissue from case of carcinoma of the breast in a cortisone-treated mouse dying at 6 days, showing no cellular reaction to the implant which contains no malignant cells in the portion sectioned. H. & E. × 32.



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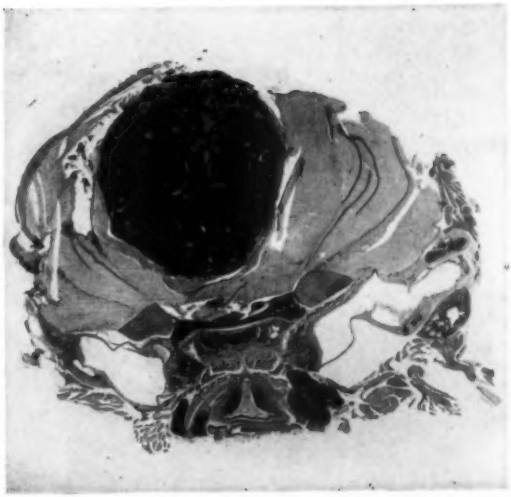

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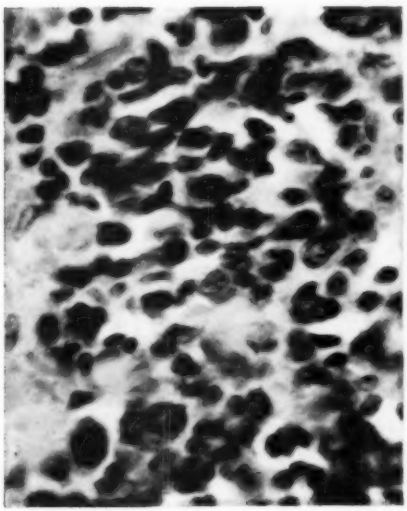

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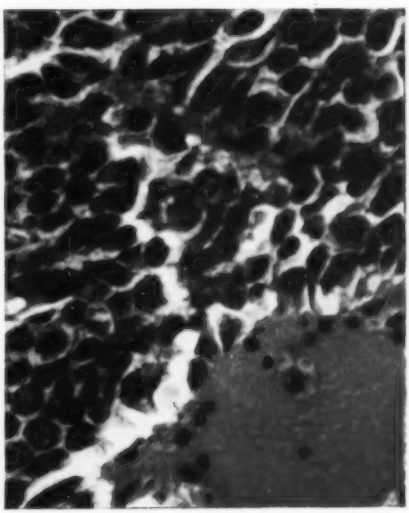

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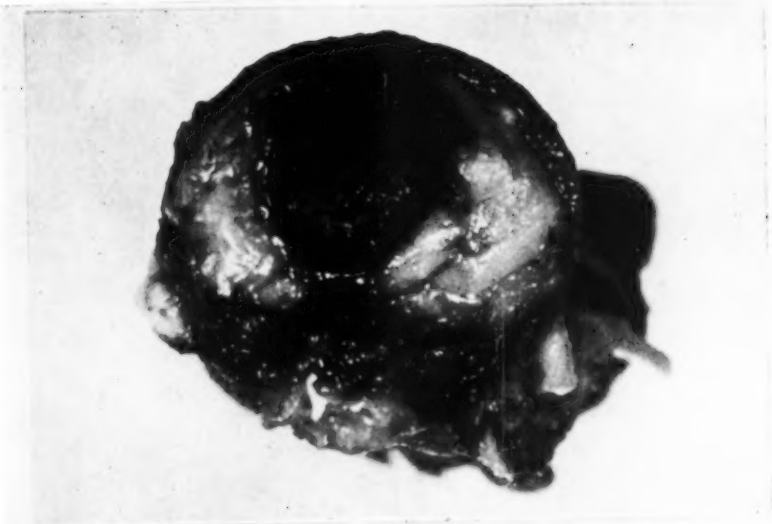

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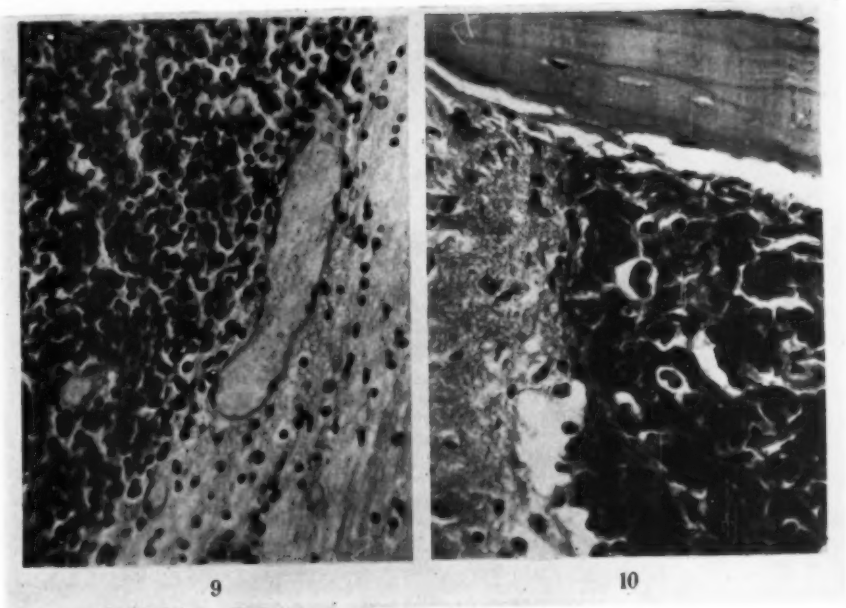

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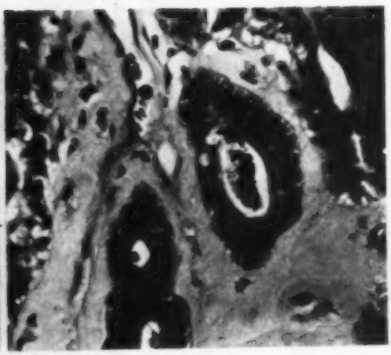

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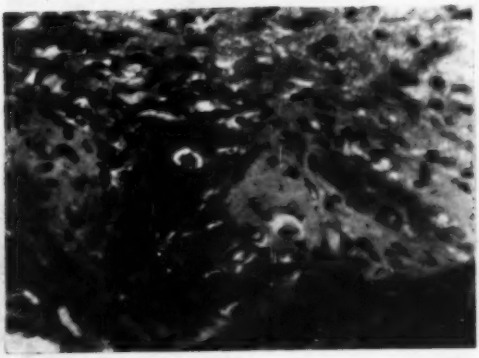

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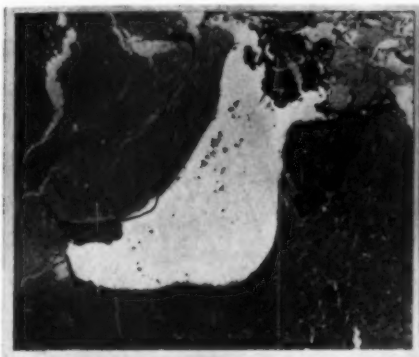

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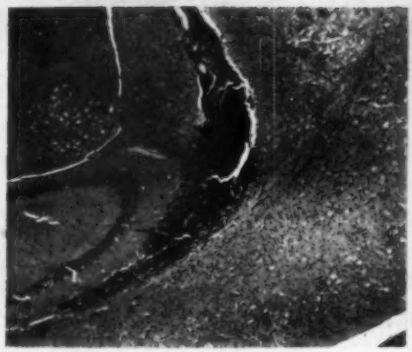

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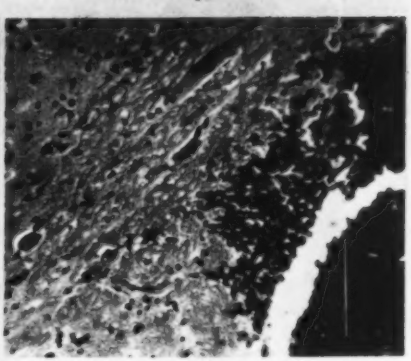

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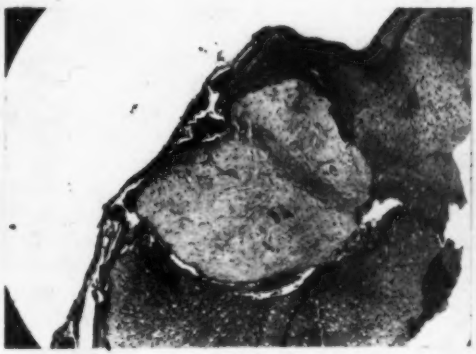

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No growth was demonstrable in mice dying or killed subsequently in these cases or from the remaining 14 human tumours, where only 6 mice (3 on cortisone) were used. These may be classified as follows :

Group 1.—Primary tumour used for implantation. No demonstrable lymph node or organ metastases. (Squamous cell carcinoma of the lung. Seminoma of the testis. Carcinoma of the breast.).

Group 2.—Primary tumour used for implantation. Histological confirmation of invaded regional lymph nodes. No demonstrable organ metastases. (Three cases of carcinoma of the breast. One polygonal-cell carcinoma of the lung.)

Group 3.—Tumour from regional nodes implanted. No demonstrable organ metastases. (Carcinoma of breast. Carcinoma of naso-pharynx.)

Group 4.—Organ metastases implanted. (Squamous cell carcinoma of lung with liver metastases (surgical biopsy.) Lymphosarcoma in a child of 7. (Post-mortem material from liver.)

Group 5.—Centrifuged deposits from the following :

(1) Pleural fluid containing malignant cells in a case of squamous cell carcinoma of the cervix, proven at autopsy. (12 mice inoculated ; 6 on cortisone.)

(2) Sternal marrow obtained after death from a case of acute leukaemia (monocytic type Naegli.)

(3) Sternal marrow from a case of Hodgkin's Disease.

Details of the three successful cases in which larger numbers of mice were used for implantation follow :

Case F.—Male, aged 50. Admitted to Harefield Hospital on September 17, 1952 with a history of lassitude since June, 1952. Signs of collapse of the left upper lobe with overlying pleurisy were present. Bronchoscopy revealed a mass of tissue blocking the left upper lobe orifice. Biopsy showed " oat cell " carcinoma. Radiotherapy was given as the growth was inoperable. Bronchoscopy on December 31, 1952, revealed no sign of the tumour and biopsy showed a few degenerate cells only. Patient returned in April, 1953, with six subcutaneous nodules, the largest of these was removed under local anaesthesia and revealed " oat cell " carcinoma.

Portions of this tumour were used for implantation. Patient died May 17, 1954, with generalised secondaries. No autopsy performed, but there were signs of secondary deposits in the brain.

Case C.—Male, aged 52. Admitted to Harefield Hospital under the care of Mr. T. Holmes Sellors with 7 weeks' history of pains in the chest and haemoptysis. X-ray revealed a mass in the left lower lobe apical section, and on September 3, 1953, a left pneumonectomy was performed. Recovery was uneventful, but on October 2, 1953, patient was re-admitted with secondary deposits and died on December 12, 1953.

The specimen consisted of a large peripheral type growth in the left lower lobe (apical section). Growth had spread to the broncho-pulmonary glands, but although the hilar glands were free, removal was probably incomplete, due to invasion of the pleura. Histology showed an " oat cell " carcinoma with tubule formation and extensive squamous metaplasia.

Case J.—Male, aged 56. Admitted to Edgware General Hospital June, 1953, under the care of Dr. G. S. C. Sowry with an opacity in the right lung. Biopsy

showed an "oat cell" carcinoma. His condition improved on radio-therapy, but in October, 1953, he was admitted to the Middlesex Hospital with deposits in the spine and numerous subcutaneous nodules over the chest and abdomen. One of these was removed under local anaesthesia and used for implantation (Fig. 7). The patient died 4 days later. Permission for a post-mortem was refused.

The results of tumour transplantation in these three cases are conveniently summarized in Table II.

TABLE II.—*Results of Tumour Transplantation.*

	Number of mice implanted.	Number surviving after 7 days.	Number of mice showing "microscopic tumours."	Number of mice showing "macroscopic tumours."
Case F.				
Cortisone treated	9	6	2 (13 & 26)	0
No cortisone	12	10	0	1 (128)
Case C.				
Cortisone treated	6	5	1 (56)	0
No cortisone	6	5	0	0
Case J.				
Cortisone treated	12	12	1 (43)	0
No cortisone	12	10	0	1 (66)

The figures in brackets refer to the number of days at which growth was observed.

HISTOLOGY.

Site of growth.

Growing tumour was found in the brain substance, in the ventricles, in the choroid plexus and in one cortisone-treated mouse in the stroma of the donor tissue when the implant was not wholly within the brain substance.

Murphy and Sturm (1923), discussing conditions determining the transplantability of tissues in the brain, and working with mouse tumours transplanted to the brain of rats, guinea-pigs and pigeons, found that the growth of foreign tissue in the brain took place only when the grafted material lay entirely in the brain substance, but when it came in contact with the ventricle a cellular reaction took place with resultant destruction of the graft.

Fig. 16 and 17: Case J shows vascularized invasive growth at 43 days in a cortisone-treated mouse replacing the ependymal lining of the lateral ventricle with no surrounding cellular response.

Fig. 18 and 19: Case F shows growth invading the brain substance from the ventricle of a cortisone-treated mouse dying at 13 days after inoculation of scrapings from the cut surface of the donor tumour.

No metastases were observed at post-mortem or on histological examination of liver, lungs, and kidneys of the mice where intracranial growth was observed. Intracranial transfer of portions of the macroscopic tumours to other mice was unsuccessful. There was a delay of some hours before transfer, as both mice died from the intracranial tumours.

Type of growth, cellular reaction and vascular supply.

In 1912 DaFano investigated homologous intracerebral transplantation of animal malignant tumours and found that growth was infiltrative or expansive, or a combination of both. The stroma originated from the blood supply of the brain and a reaction of plasma cells and lymphocytes appeared only in partially immune animals.

In the present investigation infiltrative and expansive growth was found, and the morphology of the cells resembled that of the original biopsy.

Although numerous thin-walled blood vessels could be seen in the tumours it is not obvious if these were provided by the host or grew from the implanted tissue. The macroscopic tumour of Case J (Fig. 5 and 6) was surrounded by an area containing blood. Numerous thin-walled vessels in the tumour opened into this pool of blood, but no direct continuity of cerebral and tumour vessels could be seen.

In the macroscopic tumour of Case F there was no surrounding pool of blood, but there were numerous vessels in the tumour and at the periphery adjacent to the brain substance (Fig. 9). The centre of this tumour was undergoing necrosis when the mouse died on the 128th day after implantation.

Vessels were seen in the growths at 56 days (Case C) and 43 days (Case J).

There was no cellular reaction around the macroscopic or the microscopic tumours at 56 days (Case C) and 43 days (Case J). At 26 days (Case F) there was a mild reaction round the graft of mononuclear cells, lymphocytes, microglial and gitter cells, and occasional plasma cells.

This cellular reaction was not as marked as that seen around some of the implanted tissue from cases of breast cancer and in two mice dying on the 11th day (Case F) where cortisone was not started until 5 days after implantation.

Portions of mouse hair and fragments of skull bone implanted with the tumour were sometimes observed (Fig. 10).

DISCUSSION

One of the major difficulties of intracranial transplantation is in the choice of suitable material from the tissues submitted.

Franks (1953) transplanting animal tumours to the guinea-pig eye found that with solid tumour transplants there was almost always central necrosis and a very severe inflammatory reaction with a polymorphonuclear exudate. With cell suspensions or ascites tumour cells the initial inflammatory reaction was much less marked.

Greene (1953) reporting the successful heterologous transplantation of thirteen cases of lung cancer stated : " The point to be emphasized is that the incidence of 'takes' in the first-generation passage of a heterologous tumour reflects its content of desmoplasia and the experience of the operator rather than any essential character of the neoplastic cells. From this point of view a single ' take ' is as expressive of the true nature of the tumour as a 100 per cent incidence and unless a complete uniformity of transplanted fragments with respect to desmoplasia and parenchyma is assured, a tabulation of the incidence of ' takes ' is not subject to meaningful interpretation."

In the present series growth has been obtained by using each of the techniques

outlined previously, but the original tumours in each case contained a large proportion of malignant cells relative to the supporting stroma.

The result in tumours containing more stroma can be illustrated in a case of carcinoma of the breast, where the fragments of tissue adjacent to those used for implantation showed roughly equal proportions of stromal and parenchymatous areas. One of the mice dying at 6 days showed the implanted material to consist of a mass of stromal fibrous tissue with no tumour cells in the portion sectioned (Fig. 20). Supporting tissue appeared to survive in the brain for a considerable number of days after implantation.

Some of the failures may be due to the fact that frozen sections were not done to assist in the choice of suitable material for implantation. In addition it was not always possible to locate the original implanted tissue or scrapings, and serial sections were not employed when nothing was seen in the operation area.

The survival rate of the mice was also a limiting factor. Only a few died during the operation, and these were replaced if enough tumour material was available. Some mice had to be killed or died in the first 14 days after implantation from trauma, haemorrhage, infection or hydrocephalus. After this somewhat stormy convalescence, no gross neurological signs were noticed except in one mouse (Case J), which showed paralysis of the left hind leg on the 59th day after intracerebral tumour inoculation. This mouse died 7 days later from the effect of the " human " tumour growing and compressing the brain and distending the skull.

Although it appears from the table that there is an increased incidence of microscopic growth in the cortisone-treated animals, the number of surviving animals was too small to assess the effects of the hormone on the growth of the implant or the cellular response of the host.

Material from cases of lung cancer appears to grow more readily than other tumours.

This has been the finding of Greene (1953), who postulates that human tumours which can be grown in animals are autonomous, whilst those which do not grow are in a dependent phase. The explanation may however be related to the host tissue rather than the tumour, and it is interesting that lung tumours have a peculiar tendency to metastasize to the brain.

In the three successful growths reported here, Case F died within 2 years of first admission to hospital, and Cases C and J within 6 months.

Case F and Case J had received radiotherapy before the appearance of the secondary subcutaneous nodules used for animal implantation.

To summarize, it is apparent that in view of the results obtained in these experiments and in those of other workers, heterologous transplantation using techniques at present available, does not appear to be a practical routine test for the biological activity of any given tumour, but it may provide a method for propagating certain human tumours in laboratory animals for experimental purposes.

SUMMARY.

Material from 20 human tumours has been implanted intracranially into a series of 180 mice.

No growth of the implanted tissue could be demonstrated in 17 of the 20 groups of mice.

In the remaining 3 cases of metastatic " oat cell " carcinoma of the lung infil-

trating growth was obtained between 13 and 128 days in 6 of the mice implanted.

One mouse each from 2 of these cases died 66 and 128 days after implantation from the effects of a large expanding intracranial vascularized " human " tumour. On section these occupied about half the width of the normal mouse skull and on microscopy showed malignant cells with numerous mitoses resembling the original biopsy.

Some of the animals were treated with cortisone in an attempt to increase the number of successful implants ; but there is insufficient data on the small number of animals surviving to assess the effects of the hormone. Growth was obtained in animals treated with cortisone as well as in normal animals.

Part of the expenses of this investigation has been defrayed by the British Empire Cancer Campaign. Cortisone was received from the Medical Research Council and from Messrs. Merck and Company, Rahway, New Jersey, U.S.A.

I wish to express my thanks to the following : Professor R. W. Scarff and Dr. A. C. Thackray for their encouragement and help ; Dr. J. B. Walter for many helpful suggestions and for providing some of the material used ; to Dr. L. M. Franks for placing at my disposal his modification of the Bashford needle ; to Mr. E. V. Willmott, F.R.P.S. for some of the photographs, and to Mr. H. A. Barker, Mr. E. C. Clayden and Mr. A. E. Cooper for unlimited and invaluable technical assistance.

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THE ACTIVITY OF THE ENZYMES SULPHATASE AND β-GLUCURONIDASE IN THE URINE, SERUM AND BLADDER TISSUE.

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THE fact that cancer of the bladder appears to be produced in men by external chemical agents used as dyestuff intermediates, and that bladder cancer can be produced in animals by a number of chemical substances, suggests that some bladder cancers in men, which are now considered to be spontaneous, are due to unidentified chemical carcinogens derived either from the environment or from the metabolic processes of the organism itself.

The incidence of cancer of the bladder is very high amongst men working in the chemical industry who have had contact with β-naphthylamine, α-naphthylamine or benzidine. Such tumours occurring in chemical workers are similar to those which occur in the general population not exposed to any known hazard.

Patients with papillary tumours of the bladder which have arisen spontaneously are liable to develop multiple tumours throughout the renal tract, either simultaneously or over a period of some years, in spite of any treatment that they have been given. These tumours occur in those parts of the renal tract where urine remains for some time, such as the lower end of the ureter, the pelvis of the kidney, the bladder, and, if present, a diverticulum. Tumours are rarely found in the upper third of the ureter, in the posterior urethra or the anterior urethra. In addition to these multiple tumours, areas of epithelial hyperplasia or even pre-malignant changes in the mucosa in such bladders are often seen. The distribution of these changes suggests that the mucosa has been exposed to a hyperplastic or carcinogenic agent present in the urine.

The symptomatology of some bladder tumours suggests that there may be a phase of irritation preceding the appearance of a tumour. A patient may be treated for an abacterial cystitis for a year or two before a tumour is recognised. The cause of this " cystitis " may be a chemical rather than a bacterial irritant.

Cases have been recorded where, following transplantation of the ureters, tumours appear to have regressed spontaneously, and diversion of the urine may have played a significant part in the regression. Such patients might have a metabolic pattern analogous to an " inborn error of metabolism " or other cause such as vitamin deficiency associated with excretion of a carcinogenic agent.

The investigations of Hueper and Wolfe (1937), of Hueper, Wiley and Wolfe (1938), those of Bonser (1943) and of Bonser, Clayson, Jull and Pyrah (1952) and others have shown that 2-amino-1-hydroxynaphthalene and some other *ortho*-aminophenols can induce bladder cancer in mice and in dogs. Among the sub-

stances present in human urine are two *ortho*-aminophenols, 3-hydroxykynurenine and 3-hydroxyanthranilic acid, which are known metabolites of tryptophan (Fig. 1). One of the chemical reactions involved in the production of these substances is similar to the conversion of β-naphthylamine to the carcinogenic 2-amino-1-hydroxynaphthalene.

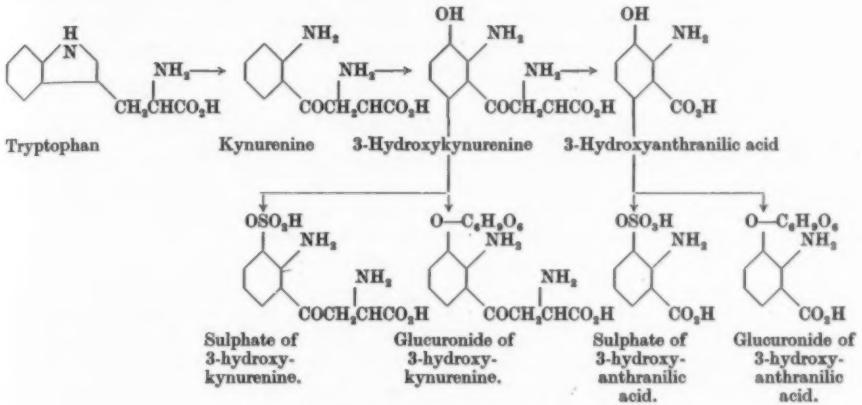

FIG. 1.—The metabolisation of tryptophan.

Aminophenols are usually excreted as sulphuric esters or as glucuronides in the urine, and these compounds would be expected to be inactive as carcinogens. The effective aminophenol concentration in urine would therefore depend upon :

(1) The total concentration of *ortho*-aminophenols and their conjugates.

(2) The time during which the urine remained in the bladder during which the aminophenols could be liberated from conjugates by action of urinary enzymes.

(3) The activities of the enzymes β-glucuronidase and sulphatase which would liberate the free *ortho*-aminophenols from their conjugates. The enzyme activities will depend on the enzyme concentrations, the pH of the urine, and the concentrations of the enzyme inhibitors or activators.

In examining this hypothesis we propose to investigate :

(1) The carcinogenic activity of the *ortho*-aminophenols and other substances found in the urine of normal subjects and of patients with cancer of the bladder.

(2) The amounts of individual *ortho*-aminophenols and their conjugation products present in normal and pathological urines.

(3) The activities of certain hydrolysing enzymes in these urines.

The following is an account of the estimation of the activities of the enzymes sulphatase and β-glucuronidase in blood and urine of normal subjects and in blood serum and bladder tissues of patients suffering from malignant disease.

EXPERIMENTAL.

Urine from hospital patients and normal individuals was collected in vessels containing small volumes of benzene as preservative. Individual specimens or 24 hour specimens were measured, and in the later investigations specific gravity

and pH were determined. The samples were centrifuged at 450 g. for 15 minutes and the deposit and supernatant urine examined separately. The deposit was re-suspended in water for determination of the enzymic activity, which was expressed in units per ml. of deposit.

Determinations on sera were made using specimens diluted tenfold with water. The enzyme activity of tissues was determined on homogenates prepared by grinding the tissue with water in glass homogenizers. The amount of tissue present was estimated by measuring the volume of the deposit, separating from an aliquot of the homogenate, on centrifugation.

Estimations of Sulphatase Activity in Urine and Serum.

The method was essentially that of Huggins and Smith (1947), modified by Robinson, Smith and Williams (1951) and Roy (1953). Trichloracetic acid solution was used as a deproteinizing agent in place of phosphotungstic acid in order to avoid the blue colour produced by the latter reagent.

Urine or other material (1 ml.), acetate buffer (0·5 M, pH 5·8, 1 ml.) and 0·05 M 4-nitrocatechol sulphuric acid (1 ml.) were incubated in a stoppered tube for 18 hours at 37°. After incubation, 1 ml. of 5 per cent trichloracetic solution was added and the mixture centrifuged for 15 minutes at 600 g.. Freshly made 0·2 per cent quinol in 2·5 N NaOH and 0·4 M Na_2SO_3 (1 ml.) was then added and the tube allowed to stand for 15 minutes. Assays were run in duplicate and a blank was set up omitting the urine during incubation and adding it just before the trichloracetic acid solution. The solutions were read against the blank in a Unicam S.P. 500 spectrophotometer (520 mμ.) calibrated with solutions of nitrocatechol. A unit of activity was that amount which liberated 1μg of nitrocatechol per hour of incubation.

The Estimation of β-Glucuronidase Activity in Urine and Serum.

The method used was essentially that of Talalay, Fishman and Huggins (1946), modified in that the substrate solution was prepared using 10 per cent ethanol. Phenolphthalein mono-β-glucuronic acid (0·05 g. Sigma) was dissolved in 10 ml. ethanol (freshly re-distilled from potassium hydroxide) and diluted to 100 ml. with water. The ethanol stabilises the phenolphthalein glucuronic acid in solution.

Urine (1 ml.), acetate buffer (1 ml. 0·1 M, pH 4·5) and substrate solution (1 ml.) were incubated in a stoppered tube for 18 hours at 37° in a waterbath. A blank containing no urine was also incubated. On removal from the waterbath, 1 ml. of urine was added to the blank and glycine buffer (1 ml. 0·4 M, pH 10·45) was added to each tube. The tubes were centrifuged at 600 g. for 15 minutes and the duplicates read against the blank on a Unicam S.P. 500 spectrophotometer at 550 mμ. The activity was expressed in units; 1 unit liberating 1 μg. phenolphthalein per hour at 37°.

Stability of the Enzymes in Urine.

Specimens of urine were stored at room temperature and in the refrigerator (0–5°C), and the activities determined after various intervals. The results (Table I) indicate that little loss of activity occurs on keeping for 6 days at room temperature or in the cold.

TABLE I.—*Activity of Enzymes in Urine at Different Times after Collection.*

No.	Temp.	β-Glucuronidase Units per ml.					Sulphatase Units per ml.				
		1 day.	3 days.	6 days.	10 days.	20 days.	1 day.	3 days.	6 days.	10 days.	20 days
1	0–5	0·35	0·35	0·32	0·25	—	0·68	0·68	0·63	0·58	—
2	0–5	1·67	1·66	1·62	1·51	—	1·12	1·12	1·12	1·02	—
3	20–25	1·10	1·11	1·00	0·78	0·35	0·64	0·62	0·60	0·46	0·27
4	20–25	2·17	2·26	—	1·92	1·03	0·90	0·90	—	0·80	0·42

Variation of Enzyme Activity with pH.

The sulphatase activity of specimens of serum was determined over the range 4·5–6·5 in 0·17 M acetate buffers. The results show (Fig. 2) that the optimum pH for both urinary and serum sulphatase is approximately 5·8 under these experimental conditions.

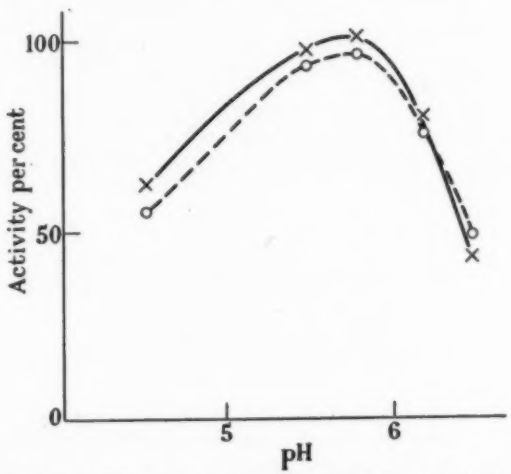

FIG. 2.—Variation of the activity of sulphatase with pH. The extinction coefficients of the solutions are plotted against pH. ×——× Urinary sulphatase. ○ - - - - ○ Serum sulphatase.

The activity of the urinary and serum β-glucuronidase was investigated over the pH range of 3·8–5·2. In both cases the maximum activity was at pH 4·5 (Fig. 3), in agreement with the pH optimum obtained by Talalay, Fishman and Huggins (1946) for β-glucuronidase from different animal tissues.

Inhibition of Activity by Urine.

Huggins and Smith (1947), Tanaka (1938), Dzialoszynski (1950) and Robinson, Smith, Spencer and Williams (1952) have shown that sulphatase is inhibited by sulphate, phosphate, sulphite, oxalate, fluoride, cyanide and certain metal ions, although the inhibition may vary with the substrate used. Some normal urinary

constituents might, therefore, be expected to inhibit sulphatase, and this has been investigated.

Urines from normal men and cancer patients were diluted with water in one series and boiled urine in another to 1/2, 1/4, 1/8 and 1/16 dilution and the sulphatase activity of 1 ml. of each of these solutions was estimated. When activity was plotted against dilution, the urine diluted with water gave a curve, whereas that diluted with boiled urine gave a straight line, presumably because the concentration of inhibitor decreased with the urine concentration in the first instance, but remained constant in the second. In a number of such experiments the inhibi-

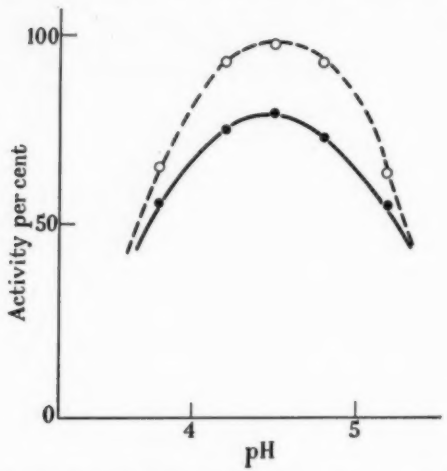

FIG. 3.—Activity of β-glucuronidase at different pH values. ○ - - - ○ Urinary β-glucuronidase. ●——● Serum β-glucuronidase.

tion of sulphatase was of the order of 20 per cent in the urines of both normal and cancer patients (Fig. 4).

The relationship between urine dilution and β-glucuronidase activity was linear (Fig. 4), indicating that urine did not inhibit β-glucuronidase.

Variation of Enzyme Activity with Time of Day.

A series of experiments was performed in which urine specimens were collected separately throughout the 24 hours. The sulphatase and β-glucuronidase activities were estimated on each specimen separately and considerable variation in both enzymes was found (Fig. 5). Similar changes in activity of the enzymes occurred in control subjects and in bladder cancer patients. Because of these variations, 24-hour specimens were collected, and all urine samples used in subsequent experiments were aliquots of such specimens.

The serum sulphatase and β-glucuronidase were estimated at different times throughout the day. The variation in activity of the serum is much less than that in the urine (Fig. 6), but for subsequent investigations blood specimens were taken at mid-day to minimise variation arising from this effect.

Investigation of Contaminants Present in Urine.

(1) *Blood and epithelial cells.*—Urine specimens were collected over a period of 24 hours, and the numbers of red and white blood corpuscles and of epithelial cells present were counted, and β-glucuronidase and sulphatase activities were

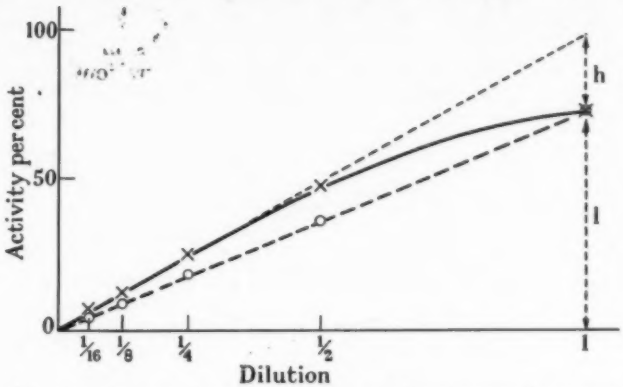

FIG. 4.—Inhibition of urinary sulphatase activity by substances normally present in urine. ×——× Urine diluted with water. ○ - - ○ Urine diluted with boiled urine of the same sample. - - - - Theoretical values of uninhibited sulphatase.

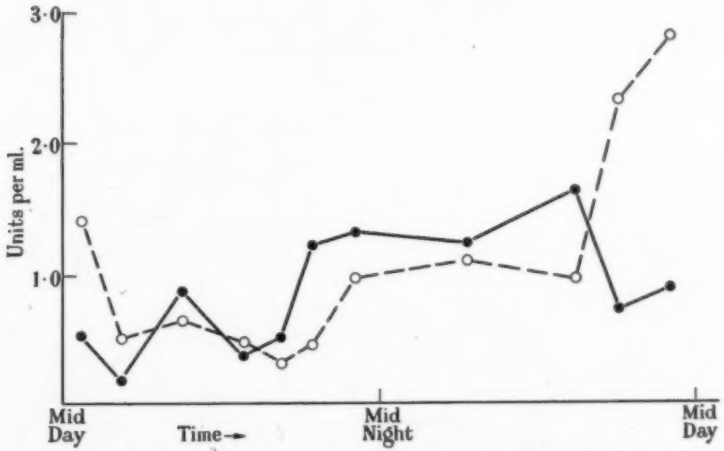

FIG. 5.—The variation of urinary sulphatase and β-glucuronidase activity throughout the 24 hours. ○ - - - ○ Sulphatase activity. ●——● β-glucuronidase activity.

estimated on the same specimens (Fig. 7). The number of cells and the enzyme activities appear to vary independently, except that the highest values for glucuronidase coincide with peaks in red cell counts. As Fishman, Springer and Brunetti (1948) found that human erythrocytes contained little or no β-glucuronidase, this

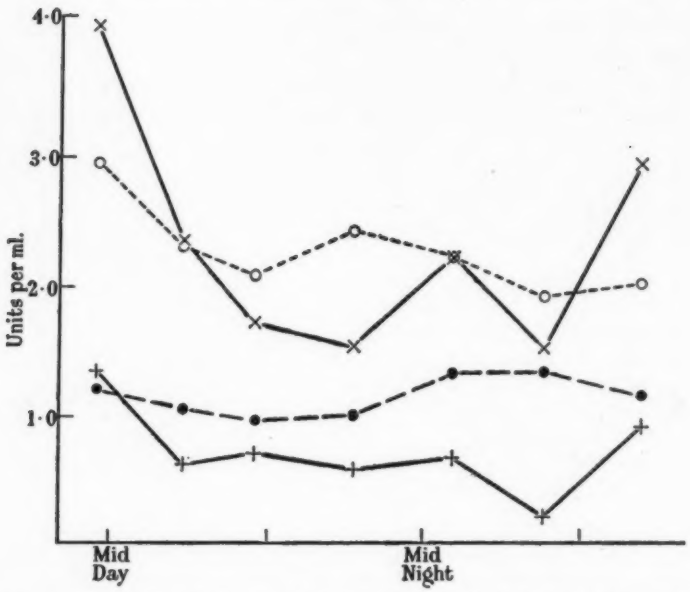

FIG. 6.—Comparison between serum and urinary β-glucuronidase and sulphatase activity throughout the 24 hours. Enzyme activity per ml. is plotted against time of day. ×——× Urinary sulphatase activity. ○ - - - ○ Serum sulphatase activity. +——+ Urinary β-glucuronidase activity. ● - - ● Serum B β-glucuronidase activity.

FIG. 7.—Variation of the sulphatase and β-glucuronidase activity of urine and of red blood corpuscles and epithelial cell content throughout the 24 hours. ▲——▲ Urinary glucuronidase units/ml. ○ - - ○ Sulphatase units per ml. ●——● Red blood corpuscles × 10^5 per ml. △——△ White blood corpuscles × 10^4 per ml. × - - × Epithelial cells × 10^4 per ml.

may be due in part to serum passed into the urine with the cells. In two cases of sterile pyuria the urinary enzymes were within the normal range.

(2) *Infection.*—The urinary β-glucuronidase and sulphatase activities of a number of patients free from malignant disease but with infected urine have been estimated (Table III). In these the β-glucuronidase values lie within the range of non-infected normals, but the figures for sulphatase are more variable. Infection is unlikely to play any significant part in the increased β-glucuronidase activity of urines from bladder-cancer patients, but it may contribute to the increased sulphatase activity.

Subjects Free of Malignant Disease.

Enzyme activities of urines from non-cancer patients, shown in Tables II and III, fall within the range 0·09–0·83 units β-glucuronidase per ml. The highest value, 0·83 (for No. 23, Table III) was in a case with an abnormally low volume (500 ml.) for the 24-hour specimen. The " normal range " has, therefore, been considered as 0·09–0·60 units per ml. urine. The normal non-infected sulphatase range is 0–0·82 units per ml., but some of the infected non-malignant cases fall outside this range.

Patients with Cancer of the Bladder.

The urinary enzyme activities of 22 patients with cancer of the bladder with sterile urine have been investigated (Table IV). The β-glucuronidase activities of 4 of these patients (1, 2, 3 and 4) lie within the normal range, but Cases 1, 2, 3, 5 and 7 had their tumours removed, either by diathermy or by X-rays, before the specimens were collected. The sulphatase activity of 7 of these cases lies within the normal range.

Patients with Active Tumours of the Bladder.

Table V shows results from 43 patients having single tumours, and Table VI shows results from 45 patients having multiple tumours. One patient in the single

TABLE II.—*Urinary Enzyme Activities of Patients Free from Malignant Disease but with Infected Urines.*

No.	Sex.	Age.	Volume/24 hours	Sulphatase Units/ml.	Glucuronidase Units/ml.	Nature of Disease.
1	M	76	1500	0·34	0·12	Prostatic adenoma
2	M	55	2200	0·85	0·17	" "
3	M	65	1300	1·5	0·18	" "
4	M	78	2700	0·49	0·18	" "
5	M	58	2350	0·60	0·21	—
6	M	54	2400	0·60	0·22	—
7	M	62	1400	0·34	0·31	Prostatic adenoma
8	M	21	1860	zero	0·33	—
9	F	28	525	0·34	0·35	Renal Tuberculosis (untreated)
10	F	53	1400	2·0	0·35	—
11	M	62	2610	1·0	0·37	—
12	M	50	1720	1·4	0·39	Prostatic obstruction
13	M	50	750	0·73	0·39	—
14	M	72	2200	1·1	0·40	—
15	M	41	4700	1·2	0·48	Prostatic calculus
16	M	82	4700	1·1	0·60	" adenoma

TABLE III.—*Urinary Enzyme Activities of Patients Free from Malignant Disease and with Sterile Urines.*

No.	Sex.	Age.	Volume/24 hours.	Sulphatase. Units/ml.	Glucuronidase. Units/ml.	Urine. pH
1	F	48	1732	0·41	0·09	—
2	M	29	1660	0·41	0·09	—
3	F	50	1572	0·82	0·11	—
4	M	50	2000	0·26	0·11	—
5	M	31	2010	0·32	0·12	—
6	M	35	1450	0·67	0·14	—
7	F	50	2000	0·07	0·15	—
8	F	62	1212	0·82	0·16	—
9	M	42	1920	0·80	0·18	—
10	M	22	1300	0·40	0·18	—
11	M	52	2010	0·20	0·18	—
12	M	25	1375	0·43	0·19	—
13	F	61	1800	0·28	0·20	5·5
14	M	62	2350	zero	0·22	—
15	M	22	1600	0·48	0·24	—
16	F	55	1570	0·65	0·27	—
17	M	50	800	0·36	0·27	5·2
18	F	64	2100	0·22	0·29	5·3
19	M	30	2800	0·08	0·30	6·7
20	F	25	1400	0·63	0·36	—
21	M	58	1400	0·30	0·45*	—
22	M	55	1100	0·35	0·47	—
23	M	47	1550	0·04	0·51*	—
24	M	32	1700	0·32	0·56	7·0
25	F	53	500	0·32	0·83	5·5

* Patients with sterile pyuria.

TABLE IV.—*Urinary Enzyme Activities of Patients with Bladder Cancer but having Sterile Urines.*

No.	Sex.	Age.	Volume/ 24 hours.	Sulphatase Units/ml.	Glucuronidase. Units/ml.	Urine. pH.	Nature of Treatment.
1	M	75	1200	0·58	0·36	5·3	—
2	M	57	2175	1·5	0·39	—	Tumour removed (Diathermy)
3	M	71	930	2·8	0·48	—	Tumour removed X-Ray
4	M	62	1500	0·56	0·58	6·5	—
5	M	40	1725	0·66	0·66	—	Tumour removed (Diathermy)
6	M	28	2000	0·13	0·70	5·3	—
7	M	50	5250	1·0	0·79	—	Tumour removed (Diathermy)
8	M	56	1800	2·9	0·93	—	—
9	M	50	2100	1·8	0·99	—	—
10	M	62	1250	0·90	1·2	6·1	—
11	M	68	1850	0·70	1·3	—	—
12	M	49	2800	0·69	1·4	6·7	—
13	M	67	2040	4·1	1·5	—	—
14	M	68	2970	1·1	1·5	—	—
15	M	49	2800	0·40	1·5	5·8	—
16	M	42	1860	1·9	1·7	—	—
17	M	58	2410	—	1·7	—	—
18	M	83	1800	0·62	1·7	—	—
19	M	36	3500	1·2	1·7	5·4	—
20	M	40	2600	1·0	2·2	6·3	—
21	M	66	1550	3·7	2·4	—	—
22	M	55	2970	1·7	3·2	—	—

TABLE V.—*Enzyme Activities of Patients with Single Tumours of the Bladder.*

No.	Sex.	Age.	Volume/ 24 hours.	Urine sulphatase. Units/ml.	Urine glucuronidase. Units/ml.	Sediment sulphatase. Units/ml.	Sediment glucuronidase. Units/ml.	Urine pH.
1	M	62	1730	3·0	0·49	3·5	2·3	—
2	M	39	2200	1·4	0·6	—	—	—
3	M	56	3750	0·47	0·6	—	—	6·7
4	M	87	2500	0·83	0·66	—	—	—
5	M	64	2450	2·1	0·7	38·0	29·0	—
6	M	67	1700	0·24	0·7	—	—	5·3
7	F	70	2650	0·18	0·74	—	—	9·7
8	F	67	800	1·58	0·86	—	—	9·1
9	M	68	710	1·2	0·9	—	31·5	—
10	M	71	2800	0·26	1·1	—	—	5·3
11	M	56	3600	0·24	1·1	—	—	5·5
12	M	68	720	1·4	1·2	14·5	10·0	—
13	M	61	900	0·87	1·2	—	—	6·0
14	M	63	1200	0·5	1·2	—	—	—
15	M	52	2100	1·0	1·3	—	—	—
16	M	54	2000	0·5	1·3	—	—	8·8
17	F	52	2110	1·7	1·3	—	—	—
18	F	69	1300	0·36	1·3	—	—	—
19	M	72	800	0·42	1·3	—	—	5·4
20	F	81	890	0·17	1·3	9·7	78·8	—
21	M	39	2680	1·2	1·4	—	10·8	—
22	M	76	1800	0·44	1·4	—	—	—
23	M	50	4300	0·8	1·5	—	—	5·0
24	M	71	2500	0·25	1·6	—	—	5·5
25	M	49	2850	0·11	1·6	—	—	—
26	F	67	750	0·84	1·6	—	—	5·4
27	M	78	2100	0·46	1·63	—	330·0	5·0
28	F	62	2800	0·26	1·7	—	—	6·4
29	M	61	1250	0·91	1·8	14·0	24·3	—
30	M	56	1200	1·7	1·8	50·0	18·3	—
31	F	69	2700	0·77	1·9	—	—	—
32	M	72	2100	1·5	2·0	—	33·0	—
33	F	69	2800	0·27	2·0	—	—	—
34	M	49	2000	0·23	2·1	—	—	—
35	M	56	2010	2·1	2·1	23·0	4·2	—
36	M	78	800	0·51	2·3	—	—	6·8
37	M	61	1100	0·6	2·4	—	—	6·4
38	M	55	3500	2·5	3·1	172·0	291·0	—
39	M	72	2600	2·4	3·2	—	—	—
40	M	61	2600	0·56	3·5	—	—	5·3
41	M	61	2320	2·1	4·2	—	—	—
42	M	59	3700	2·0	4·8	34·0	66·0	—
43	M	50	1900	0·8	5·2	—	—	5·4

tumour group and 2 in the multiple group have β-glucuronidase values within the normal range, but in the latter cases both have abnormally high 24-hour urine volumes (7·5l.–3·5l.). Many sulphatase values fall within the normal range although most of the values are above the highest of the normal values.

Patients without Active Tumours of the Bladder.

All of the 15 patients who had new tumours over a period of 1–8 years after destruction or removal of the original tumours had β-glucuronidase values above the normal range (Table VII). Of the figures for sulphatase activity in this series, 9 fell within the normal range.

Table VIII shows results obtained from urines of 16 patients who have had tumours of the bladder removed and have developed no fresh tumours for some

TABLE VI.—*Urinary Enzyme Activities of Patients with Multiple Tumours of the Bladder.*

No.	Sex.	Age.	Volume/ 24 hours.	Urine sulphatase. Units/ml.	Urine glucuronidase. Units/ml.	Sediment sulphatase. Units/ml.	Sediment glucuronidase. Units/ml.	Urine pH.
1	M	51	7500	2·5	0·25	31·0	8·7	—
2	M	56	3500	0·85	0·60	7·8	29·4	—
3	M	54	2550	0·50	0·63	—	—	5·4
4	F	77	740	2·5	0·64	35·0	15·0	—
5	M	71	2010	2·5	0·75	76·0	—	—
6	M	54	2650	0·91	0·76	16·0	82·0	—
7	M	65	3700	1·5	0·82	8·0	4·8	—
8	M	54	2020	1·5	0·86	18·0	10·1	—
9	M	71	2100	1·4	0·90	—	—	—
10	M	65	1280	2·5	0·90	35·0	27·0	—
11	M	71	2430	1·5	0·90	65·0	48·0	—
12	M	46	2120	1·3	0·94	8·0	4·6	—
13	M	62	2450	1·0	0·94	—	—	—
14	M	68	800	1·1	1·1	—	—	—
15	M	78	1800	1·5	1·2	—	—	8·3
16	F	74	1800	0·42	1·3	—	—	6·0
17	M	—	2040	4·5	1·35	145·0	5·3	—
18	M	71	3100	2·1	1·5	—	—	5·4
19	M	66	2200	1·3	1·5	37·0	44·0	—
20	M	73	2500	1·5	1·6	—	—	—
21	M	59	6200	0·72	1·6	—	—	5·5
22	M	50	1300	2·2	1·6	—	—	6·7
23	M	68	1000	1·8	1·7	—	—	—
24	F	74	1600	0·48	1·8	—	—	6·7
25	M	73	2100	2·5	1·8	20·0	3·8	—
26	M	54	2450	2·3	1·8	34·0	13·9	—
27	M	67	2400	2·2	1·9	35·0	—	—
28	M	45	1000	0·52	1·9	—	—	5·3
29	M	73	2800	1·9	2·0	—	—	—
30	M	57	2100	1·7	2·1	—	—	—
31	M	71	1700	0·46	2·1	—	—	6·7
32	M	50	1470	2·0	2·2	51·5	27·8	—
33	M	45	2100	0·75	2·3	—	—	5·3
34	M	63	2000	0·72	2·3	39·0	75·0	5·5
35	M	50	1630	2·5	2·3	125·0	11·9	—
36	F	62	1030	2·1	2·4	80·0	52·0	—
37	F	58	900	1·1	2·4	—	—	6·9
38	F	50	1100	2·2	2·5	—	—	—
39	M	57	3500	1·8	2·8	280·0	225·0	—
40	M	—	1470	5·0	3·9	105·0	3·4	—
41	M	71	2700	2·4	4·2	—	—	6·4

TABLE VII.—*Urinary Enzyme Activities of Patients with Recurrent Tumours of the Bladder.*

No.	Sex.	Age.	Volume/ 24 hours.	Urine sulphatase. Units/ml.	Urine glucuronidase. Units/ml	Sediment sulphatase. Units/ml.	Sediment glucuronidase. Units/ml.	Urine pH.
1	M	50	2170	3·0	0·65	115·0	—	—
2	M	42	2650	1·15	0·72	45·0	8·9	—
3	M	66	1800	0·25	0·83	—	—	7·3
4	F	38	2800	0·15	1·1	—	—	6·2
5	M	40	2750	0·31	1·3	—	—	5·5
6	F	73	400	2·0	1·6	—	—	5·3
7	M	52	2700	0·4	1·7	—	—	6·4
8	M	42	1860	1·9	1·7	—	—	—
9	F	38	2300	0·66	1·8	—	—	5·8
10	M	52	2750	0·54	1·8	—	—	6·7
11	F	75	2600	2·16	1·9	—	—	8·1
12	M	47	2750	0·58	2·3	—	—	6·7
13	M	47	1800	0·42	2·4	—	—	5·3
14	M	60	1400	2·3	2·45	43·0	2·9	—
15	F	75	1300	0·72	3·2	—	—	5·0

TABLE VIII.—*Enzyme Activities of Patients with No Active Tumours.*

No.	Sex.	Age.	Volume/ 24 hours.	Urine sulphatase. Units/ml.	Urine glucuronidase. Units/ml.	Sediment sulphatase. Units/ml.	Sediment glucuronidase. Units/ml.	Urine pH.
1	M	63	2500	2·3	0·38	28·5	6·0	—
2	M	55	2320	3·5	0·39	—	—	—
3	F	52	1600	1·7	0·58	35·0	18·3	—
4	M	61	2650	2·0	0·66	48·5	1·3	—
5	M	36	2530	2·0	0·67	80·0	—	—
6	F	85	800	1·3	0·78	42·0	26·4	—
7	M	60	1220	1·1	1·1	—	—	—
8	F	—	2780	1·5	1·3	13·5	5·4	—
9	F	74	2300	0·23	1·4	—	—	5·3
10	M	55	2500	2·1	1·8	—	—	—
11	M	64	1600	0·33	2·0	—	—	9·1
12	M	71	1500	0·9	2·1	—	—	—
13	F	—	1400	2·5	2·1	—	—	—
14	F	52	2600	0·54	2·3	—	—	5·1
15	M	66	2100	0·8	2·5	—	—	—
16	M	60	1440	2·0	2·6	25·0	0·9	—

years. In this group, 3 cases have β-glucuronidase values within the normal range, and 4 have sulphatase values within the normal range.

Enzyme Activity of Ureteric Urine.

In 6 cases of bladder cancer, specimens of urine taken from the ureters by indwelling polythene catheters were examined (Table IX). In most of these cases the β-glucuronidase and sulphatase activity was above the normal range, indicating that these urinary enzymes do not originate entirely from the bladder. When samples were taken from both ureters at the same time, they often gave similar values which suggests that the enzymes are not derived from the kidney, but rather from the circulating blood.

Patients with Cancer of Sites Other than the Bladder.

Results from 22 cases with cancer of sites other than the bladder are shown in Table X. In this group, 8 cases are above the upper limit of the normal range for β-glucuronidase, but in one case (No. 16, carcinoma of lung) the urine volume for the 24-hour specimen was very low. Of the 5 cases of cancer of the testes, 4 had high β-glucuronidase, and of 3 cases of prostatic cancer, 2 had high values. The only specimen examined from a patient with cancer of the stomach had high β-glucuronidase activity. More patients of these types will be examined. Eleven of the sulphatase results are above the upper limit of the normal range.

Cases of Tuberculosis.

The urinary sulphatase activities of patients suffering from pulmonary tuberculosis and the urinary β-glucuronidase of patients with renal tuberculosis (Table XI) were high. All these patients were under treatment with *para*-aminosalicylic acid at the time of the investigation.

Enzyme Activity in Urine Sediments.

Tables V–VIII include figures for the enzyme activity associated with the urine deposits. The results vary greatly from one specimen to another, but in

TABLE IX.—*Enzyme Activity of Urine taken from Ureters.*

No.	Sex. Age.	Date of specimen.	Volume per 24 hour.	Glucuronidase. Units/ml.	Sulphatase. Units/ml.	Ureter.	Operation and date.
1	F, 57	5.v.54	160	1·37	0·57	Both	Cystectomy 19.iv.54.
		9.v.54	1000	0·15	0·13	,,	
2	F, 80	9.ii.54	800	1·05	1·2	Right	Ureterostomy and nephrectomy 8.i.54.
3	M, 76	9.iv.54	1800	1·4	0·44	Pre-operative	Partial cystectomy
		20.iv.54	1100	0·35	—	Left	11.iv.54.
			2000	0·36	—	Right	
		25.iv.54	1600	0·43	0·87	Both	
4	M, 63	10.iii.54	1200	1·2	0·37	Pre-operative	Bilateral transplantation of ureters into colon 11.iii.54.
		15.iii.54	800	1·2	0·37	Both	
5	M, 56	11.iii.54	4040	0·55	0·46	Pre-operative	Right ureteric transplantation.
		12.iii.54	4800	0·53	0·61	,,	
		15.iii.54	1400	1·2	2·5	Right	
		19.iii.54	750	1·8	0·49	,,	
			1300	0·03	1·33	Left through bladder	
6	M, 57	12.vii.54	2700	1·56	0·88	Pre-operative	Cystectomy 14.vii.54.
		13.vii.54	3500	1·32	0·66	Pre-operative	
		16.vii.54	250	0·36	1·7	Left	
			150	0·60	2·0	Right	
		17.vii.54	175	0·82	2·7	Left	
			80	2·0	2·4	Right	
		18.vii.54	510	3·1	0·57	Left	
			250	4·2	2·4	Right	
		19.vii.54	100	4·3	0·50	Left	
			240	4·9	0·36	Right	
		20.vii.54	850	5·2	2·8	Left	
			350	4·9	0·86	Right	
		21.vii.54	1000	2·6	—	Left	
			400	4·9	2·1	Right	
		22.vii.54	1100	5·4	1·9	Left	
			200	4·3	2·8	Right	
		23.vii.54	1650	2·5	1·9	Left	
			500	4·5	2·4	Right	
		24.vii.54	450	4·0	1·8	Left	
			500	4·5	1·3	Right	

general sediments show a very much higher activity of both β-glucuronidase and sulphatase than equal volumes of the corresponding urines. Cancer of the bladder is most frequently found on the lower part of the bladder in both man and animals, and the increased enzyme concentration associated with the sediment may be responsible, at least in part, for this.

pH Values of Urines.

The pH values of different specimens of urine given in Tables III, IV, V, VI, VII, VIII and X show wide variations, with no clear difference between the patients with cancer of the bladder and other patients, or normal subjects. The few alkaline urines found (Tables V, VI and VIII) were infected specimens. Most of the specimens had pH values between 5·0 and 6·0, so that the actual glucuronidase activity would be between 20 and 80 per cent of the activity of the optimal pH of 4·5.

TABLE X.—*Urinary Enzyme Activities of Patients with Cancer of Sites other than Bladder.*

No.	Sex.	Age.	Site of primary tumour.	Volume/24 hours.	Sulphatase. Units/ml.	Glucuronidase. Unit/ml.	Urine pH.
1	M	60	Larynx	1010	0·80	0·09	
2	F	43	Breast	1650	1·30	0·09	
3	F	45	Mylomatosis	1700	0·80	0·09	
4	F	68	Oesophagus	1890	0·40	0·09	
5	M	24	Myeloid leukaemia	2200	0·67	0·11	
6	F	54	Sarcoma thigh	720	0·58	0·11	
7	M	66	Sigmoid colon	1320	0·80	0·15	
8	F	54	Breast	960	0·60	0·18	
9	M	74	Prostate	1500	0·83	0·23	
10	M	47	Colon	1800	1·70	0·32	5·4
11	F	—	Larynx	650	0·90	0·33	
12	F	43	Kidney	1750	0·66	0·42	
13	F	76	Lymphatic leukaemia	1760	0·40	0·43	
14	M	37	Testicle	700	1·39	0·46	5·3
				300	1·15	0·60	5·4
15	M	29	Testicle	900	0·68	0·65	5·8
				1400	1·08	0·80	5·4
16	M	75	Lung	500	2·5	0·78	
17	M	66	Prostate	1100	0·74	0·79	
18	M	56	Stomach	1080	2·4	0·84	
19	M	39	Testicle	1870	3·5	0·98	
20	M	57	Prostate	2600	0·69	0·99	
21	M	31	Testicle	2100	1·73	1·35	6·7
				2100	1·69	1·39	7·0
22	M	18	Testicle	900	2·28	1·35	5·8
				1500	2·78	1·54	5·4

TABLE XI.—*Urinary Enzyme Activities of Patients with Tuberculosis.*

No.	Sex.	Age.	Site of disease.	Volume/24 hours.	Sulphatase. Units/ml.	Glucuronidase. Units/ml.
1	M	52	Lung	1600	0·72	0·29
2	F	43	,,	1900	1·20	0·29
3	M	41	,,	1600	0·68	0·30
4	M	49	,,	1750	1·20	0·45
5	M	32	Kidney	2500	0·35	0·60
6	M	58	,,	2520	0·80	0·63
7	F	37	Lung	1250	2·90	0·68
8	M	72	,,	1000	0·92	0·75
9	M	36	Kidney	1800	0·70	0·84
10	M	42	,,	1430	0·45	0·96
11	M	49	,,	1790	0·60	1·1
12	M	52	,,	1800	0·90	1·1

Enzyme Activity in Serum.

The enzyme activity in normal human serum (Table XII) ranged from 0·75–2·5 β-glucuronidase units per ml. and from 1·2–3·3 sulphatase units per ml. Table XIII gives results for 12 patients with single tumours and for 21 patients with multiple tumours of the bladder. There appears to be an increase in the sulphatase activity compared with the normal values. In all except 5 cases in each of the 2 groups the β-glucuronidase values are higher than the normal values.

Enzyme Activity of Malignant and Non-Malignant Bladder Tissues.

The enzyme activities of the bladder mucosa and bladder tissue tumour taken from patients at operation (Table XIV) show that both types of tissue contained

TABLE XII.—*Enzyme Activity of Serum from Patients free from Malignant Disease.*

No.	Age.	Sex.	Serum sulphatase. Units/ml.	Serum glucuronidase. Units/ml.
1	61	M	—	0·75
2	56	M	—	0·79
3	76	M	1·6	1·0
4	52	M	1·2	1·4
5	58	F	2·1	1·6
6	62	M	3·3	1·7
7	85	F	1·5	1·8
8	65	M	1·7	1·9
9	55	M	1·4	2·1
10	62	M	2·7	2·3
11	50	M	1·8	2·5
12	36	M	1·4	2·5

TABLE XIII.—*Enzyme Activity of Serum from Patients with Cancer of the Bladder.*

Patients with Single Tumours.

No.	Age.	Sex.	Serum sulphatase. Units/ml.	Serum glucuronidase. Units/ml.
1	39	M	—	1·4
2	64	M	2·6	1·5
3	40	M	—	2·0
4	72	M	—	2·0
5	61	M	2·3	2·4
6	65	M	—	2·7
7	41	M	2·9	3·3
8	71	M	—	3·8
9	68	M	3·1	4·1
10	81	F	1·9	4·8
11	52	M	1·1	6·0
12	59	M	2·2	7·5

Patients with Multiple Tumours.

1	54	M	—	2·0
2	47	M	—	2·1
3	68	M	3·2	2·1
4	66	M	—	2·1
5	82	M	1·8	2·2
6	57	M	—	2·7
7	55	M	2·4	2·8
8	57	M	3·6	2·9
9	59	M	1·5	3·0
10	57	M	—	3·0
11	54	M	2·4	3·2
12	47	M	1·7	3·3
13	57	M	—	3·5
14	46	M	—	3·6
15	67	M	2·0	3·7
16	66	M	—	4·0
17	54	M	1·65	4·8
18	42	M	—	6·4
19	73	M	—	7·9
20	73	M	2·9	10·7
21	71	M	—	10·8

TABLE XIV.—*Enzyme Activities of Bladder Tumour and Adjacent Bladder Mucosa.*

No.	Age.	Sex.	Glucuronidase (units/ml.).		Sulphatase (units/ml.).	
			Tumour.	Mucosa.	Tumour.	Mucosa.
1	73	M	4·1	—	2·1	—
2	59	M	9·4	—	116·0	—
3	63	M	12·5	12·0	17·0	8·2
4	87	M	19·0	11·0	41·0	11·0
5	39	M	25·0	9·0	23·0	2·7
6	71	M	26·0	55·7	14·6	15·0
7	61	M	29·0	61·0	54·0	10·0
8	59	M	34·0	29·0	48·0	5·2
9	50	M	43·0	35·0	41·0	11·0
10	72	M	44·0	55·0	48·0	9·0
11	72	M	47·0	25·0	107·0	10·0
12	54	M	50·0	—	25·0	—
13	69	F	55·0	—	41·0	—
14	63	M	55·0	12·0	44·0	11·0
15	42	M	58·0	—	400·0	—
16	72	M	58·0	—	41·0	—
17	71	M	72·0	55·0	32·0	15·0
18	45	M	77·0	—	50·0	—
19	64	F	79·0	—	128·0	—
20	59	M	91·0	—	40·0	—
21	69	M	96·0	73·0	83·0	12·0
22	45	M	97·0	—	7·4	—
23	67	F	106·0	61·0	—	0·6
24	57	M	112·0	40·0	46·0	16·0
25	61	M	120·0	7·5	31·0	32·0
26	56	F	140·0	105·0	78·0	16·0
27	50	M	145·0	57·0	26·0	26·0
28	67	M	156·0	—	82·0	—
29	74	M	170·0	—	116·0	—
30	58	M	176·0	—	61·0	—
31	64	F	255·0	235·0	240·0	11·0
32	71	M	455·0	—	390·0	—

β-glucuronidase and sulphatase. In most of the specimens, the tumour tissue had more enzyme activity than the corresponding normal mucosa. Fishman and Anylan (1947) found that other types of human cancer contained more β-glucuronidase than the adjacent normal tissue.

DISCUSSION.

The enzyme excretions have been expressed as activities per unit volume of urine, but the volume of the urine was always recorded, and the results have also been calculated as total units of enzyme excreted per day. When expressed in this way, the results were essentially the same except that the bladder cancer patients gave higher values because such patients are usually encouraged to take large volumes of fluid and so excrete more urine than other subjects. The method of expressing the results which is used weights the result against the difference which has been found, namely increased enzyme excretion in cancer patients.

The investigations would be facilitated if a means of measuring the increased excretion could be used which would avoid the collection of the 24-hour specimen. The ratio of enzyme activity to some urinary constituent or to the total urinary constituents, as indicated by the specific gravity, might serve this purpose. This

problem and the investigation of β-glucuronidase in sera and urines of patients with cancer of sites other than the bladder are being investigated.

The data presented show that patients with cancer of the bladder have higher urinary β-glucuronidase and sulphatase concentrations than those subjects free of malignant disease. Most of the bladder cancer patients examined excrete large volumes of urine so that the total enzyme excretion in such cases is generally larger than in other subjects. This finding is in agreement with the hypothesis that the urinary β-glucuronidase is a contributing cause of the disease, acting by liberation of carcinogenic aminophenols or other compounds from inactive conjugates. On the other hand, the raised urinary β-glucuronidase may be a result of the disease rather than the cause. In the few cases in which specimens of urine were taken directly from the ureter, the β-glucuronidase and sulphatase activities were of the same order as it is in bladder urine; the extra β-glucuronidase is unlikely to have its origin in the bladder although normal bladder mucosa usually had a higher concentration of β-glucuronidase than urine. The urinary β-glucuronidase was generally raised even in patients with no active tumour. The bladder tumours like many other malignant tumours, usually had a higher sulphatase and β-glucuronidase content than the normal tissue from which the tumour was derived.

Although the serum enzyme values of cancer patients were generally above the normal range, the distinction between the cancer and cancer-free patients was much less clear cut than was the case with the urinary enzymes. The urinary enzymes are probably derived from the blood; the amount of the enzymes in urine being dependent on the serum concentration and the degree to which protein, including enzymes, pass through the kidney. The enzymes may pass from the kidney itself directly into urine, as kidney tissue contains both β-glucuronidase and sulphatase, but the fact that the enzyme activity of urine from the two kidneys at any one time was the same would indicate that the enzymes were derived from circulating blood rather than kidney tissue.

Other work on the action of enzymes on derivatives of the carcinogenic 2-amino-1-hydroxynaphthalene (Boyland, Manson, Sims and Williams, 1955) has shown that whereas 2-amino-1-hydroxynaphthalene phosphoric ester and glucuronide are hydrolysed by phosphatase and β-glucuronidase respectively, 2-amino-1-hydroxynaphthalene sulphuric ester is not hydrolysed by the sulphatases of human urine, rat kidney or takadiastase. This would indicate that sulphatase could not play any rôle in carcinogenesis from β-naphthylamine, as the 2-amino-1-hydroxynaphthalene sulphuric ester would not be hydrolysed in urine. β-Glucuronidase can, however, release the carcinogenic 2-amino-1-hydroxynaphthalene from its glucuronide which Boyland and Manson (unpublished observations) have shown to be a metabolite of β-naphthylamine.

Although the level of the urinary enzymes may be of value in prognosis, in as much as it may indicate the liability of fresh tumours to develop, the patients who have been investigated so far over the last year, with different types of tumour and different types of treatment, have not been followed up long enough to make any definite statement as to the value of the estimations in prognosis.

SUMMARY.

(1) Urinary sulphatase activity of patients with cancer of the bladder is often high compared with normals. This rise may be due in part to infection. Urine

sediments from bladder cancer patients are always high in sulphatase activity but serum sulphatase is not raised in cancer patients.

(2) β-glucuronidase activity is almost always increased in the urine of patients with cancer of the bladder and remains high in most cases even after the tumour has been removed. The β-glucuronidase activity of urine is independent of infection of the urine. A few patients suffering from other forms of cancer had slightly increased urinary β-glucuronidase.

(3) The β-glucuronidase activity is high in all urinary sediment examined and in most sera from patients suffering from cancer of the bladder.

(4) Bladder-cancer tissue contained more β-glucuronidase and sulphatase than the corresponding bladder mucosa.

(5) The estimation of β-glucuronidase activity in urine may be useful for prognostic purposes in bladder cancer.

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NEOPLASIA AND INTERNAL ENVIRONMENT.

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REFERENCES

I. INTRODUCTION.

DURING the last fifteen years it has been generally recognised that the growth of tumours depends not only on the intrinsic properties of neoplastic cells but also on the environment in which they develop. Of the morphogenetic factors active

in post-embryonic life only the secretions of endocrine glands have been identified so far. When speaking of internal environment in connection with normal or neoplastic growth one refers to variations in hormone levels. As will be shown, the reaction to an exogenous carcinogenic agent can be profoundly altered by a hormonal deficiency. Variations in hormone level can be the essential factor for the development of some tumours and such changes can, in certain circumstances, lead to retrogression of a cancer. In Section II the recent investigations into the response of hypophysectomized or thyroidectomized rodents to chemical carcinogens will be discussed. In the third section the neoplasms found in thyroid hormone deficient rats and mice will be reviewed and their relationship to human thyroid cancers discussed. Section IV deals with retrogression of malignant growths with special emphasis on the morphology of human cancers regressing under the influence of changes in the hormonal environment.

II. The Role of Hormones in the Initiation of Chemically Induced Tumours.

Many organs cannot maintain their size in the absence of hormones on which they depend for their normal development and function. Generally it is difficult to induce neoplastic growths in atrophic tissues such as the breast of a rodent ovariectomized early in life. Only with the aid of massive doses of some of the most powerful carcinogenic hydrocarbons a pathological growth response can be occasionally obtained (Shay, Harris and Gruenstein, 1952). In the experiments to be discussed this type of atrophy can be ruled out as a major factor responsible for the failure of the carcinogen to act in its usual manner.

Moon, Simpson and Evans (1952) implanted pellets of methycholanthrene into the gastrocnemius of 15 hypophysectomized rats and saw only 1 sarcoma during the 316 days of observation. Twelve rapidly progressing cancers developed in the 15 intact controls. Griffin, Rinfret and Corsigilia (1953) fed a diet containing 3-methyl-4-dimethylaminoazobenzene to 18 hypophysectomized rats of two different strains for periods up to 19 weeks. The only pathological change observed was a mild cirrhosis in the liver of one of them. Multiple hepatomas appeared already during the 14th week in the intact controls. It is astonishing that two animals in which approximately 25 per cent of the pituitary were still present showed no signs of neoplasia in the liver but only cirrhosis. In a later communication, Richardson, Griffin and Rinfret (1953) described the histology of 24 completely and 2 incompletely hypophysectomized rats treated with the same carcinogenic azo dye. The livers of the former were normal in every respect but those of the latter showed the usual cirrhosis, proliferation of bile ducts, fatty changes and beginning neoplastic growth. Study of the adrenal glands of the experimental animals led the authors to suggest that "some factor elaborated by the pituitary is essential for adrenal-lipoid maintenance and subsequent cancer formation". However, their preliminary results with crude gonadotrophin fractions are difficult to reconcile with this contention (Griffin, Rinfret, Robertson and O'Neal, 1953).

Bielschowsky and Hall (1953) gave an account of the tumours which can be induced in thyroidectomized rats with 2-aminofluorene (AF) or its monoacetyl derivative (AAF). Only the results obtained with the amine will be quoted in detail. No liver tumour was found in 20 completely thyroidectomized rats,

whereas 6 of the 10 partially thyroidectomized animals and 16 of the 18 intact controls had hepatomas. Reduction in the incidence of liver tumours induced by AAF in rats treated with thiouracil had been reported by Paschkis, Cantarow and Stasney (1948) and by Leathem and Barken (1950). The Philadelphia group found that under the influence of the goitrogen the percentage of hepatomas in males dropped from 83·6 to 20·2 and in the females from 46 per cent to zero. Leatham and Barken repeated this experiment with pair-fed rats and thus eliminated the possible effect of reduced food intake. After 8 months 50 per cent of the animals receiving the carcinogen alone had liver cancers and the rest benign cystic cholangiomas. Only one of the 8 males receiving AAF and thiouracil developed a hepatoma, but 6 had cystic lesions in the liver. Paschkis, Cantarow and Stasney (1948) included in their paper an experiment in which para-dimethylaminoazobenzene was used as carcinogen. In the males thiouracil did not modify the action of the azo dye, but it lowered the incidence of liver tumours in the females from 88 to 46 per cent. There exists therefore evidence that thyroid hormone deficiency modifies the response of the liver cells against AF and AAF and, perhaps, against butter yellow. In the writer's experience the degree of thyroxine deficiency is crucial for the outcome of the experiment. Animals, the pituitary of which still contained acidophilic cells in appreciable numbers, always showed the typical liver lesions which follow the administration of the aromatic amine. Rats with pituitaries void of acidophils, i.e. animals in which only traces of thyroxine could still be present in the body, had livers free of neoplastic changes. It should be kept in mind that a severe degree of thyroxine deficiency implies lack of growth hormone. The endocrine imbalance is rather complex since adrenals and gonads too are affected by complete thyroidectomy. However, gonadectomy gives only a very slight protection against the hepatoma-inducing action of AAF. From the studies on tumour induction by AF and AAF in thyroxine deficient animals it became evident that the protection given by thyroidectomy was restricted to the liver, whereas the susceptibility of the sebaceous glands of the meatus acousticus externus was not affected and that of the retrobulbar glands was even increased.

Richardson, Griffin and Rinfret (1953), as well as the writer, were impressed by the absence of the expected signs of liver damage in the hypophysectomized or thyroidectomized rats treated with carcinogens which have a predelection for the liver. The amounts of the carcinogenic agents given in these experiments always induce liver damage in intact animals. In my experiments the only abnormality noted was the occasional occurrence of highly vacuolated liver cells. It is known that thyroxine deficiency modifies the reaction of the liver to dietary injury (György and Goldblatt, 1945 ; Handler and Follis, 1948 ; Sellers and You, 1951). Léger, Masson and Prado (1947) observed that removal of the thyroid increased the resistance of the rat to the egg-white reaction. No satisfactory explanation can be offered at present as to the mechanism by which thyroxine deficiency protects the liver against injury of widely different etiology. The results obtained with carcinogenic agents are in line with the old conception of a causal connection between liver cell damage and hepatoma formation, which is further strengthened by the observation that thyroidectomy performed once AAF has produced liver cell damage, does not arrest the development of neoplastic growth.

The skin seems to be an organ in which hormone deficiency does not greatly

modify the reaction to chemical carcinogens. Korteweg and Thomas (1939) studied the action of 3,4-benzpyrene on the skin of hypophysectomized mice. In one experiment the interval between operation and the start of painting the skin was 4 weeks, in another 5–7-months-old animals were used hypophysectomized at the age of 6 weeks. Skin tumours developed in all the control animals, papillomas appearing 44–100 days and carcinomas 93–104 days after onset of treatment. All of the 14 hypophysectomized mice still alive when the first papilloma was found on the 105th day of the experiment developed tumours of the skin; ten of them became cancerous between the 121st and 190th day. Although carcinogenesis was retarded in the hypophysectomized mice, the response of their skin to benzpyrene was qualitatively the same. Hall and Bielschowsky (unpublished results) repeated the work of Korteweg and Thomas (1939) using methylcholanthrene as carcinogen and restricting the application of this compound to 23 paintings. Our results are in excellent agreement with those of the Dutch workers. Benign and malignant tumours of the epidermis appeared after the administration of methylcholanthrene had been terminated in completely hypophysectomized mice showing the typical atrophy of the gonads and all the other morphological signs of pituitary deficiency. The neoplasms, however, developed slower than in the controls. One melanoma was found in a completely hypophysectomized female.

The histology of the reaction of muscle and connective tissue to methylcholanthrene in the hypophysectomized rat has not yet been described. The few data available indicate that once a hydrocarbon has induced a sarcoma, hypophysectomy will only retard its growth (Ball and Samuels, 1936). In the case of the liver of thyroidectomized rats the carcinogen apparently fails to change normal into neoplastic cells, i.e. the initiation of the neoplastic process is inhibited.

There is some suggestive evidence that other factors may be involved. Hypophysectomy seemingly does not affect the skin and its appendages to judge from the good condition of the fur and the rapid regrowth of clipped hairs in mice. Zeckwer (1953) noted that in the adrenalectomized rat regrowth of hair was even accelerated. Fraser and Nay (1953) observed a similar effect in ovariectomized mice, but inhibition of hair-growth towards the end of pregnancy, confirming earlier findings of the inhibitory action of elevated doses of oestrogen. In this connection recent results of Engelbreth-Holm and Jensen (1953) are relevant. The injection of growth hormone into mice failed to alter the incidence of skin tumours induced by a single application of 9,10-dimethyl, 1,2-benzanthracene. The only effect noted was a delayed appearance of neoplasms in the injected animals. From such observations and those of Korteweg and Thomas (1939) it seems that hormones have but little influence on the normal and neoplastic growth processes in the epidermis. On the other hand there is ample evidence that pituitary secretions can influence the growth of liver and of subcutaneous tissues as seen in acromegaly or after the injection of crude pituitary extracts.

III. The Role of Thyroid Hormone Deficiency in Tumourigenesis in Thyroid and Pituitary.

During the last decade benign and malignant neoplasms of the thyroid of rats and mice have been obtained by various means. The conditions under which they occur are : (1) After administration of goitrogenic substances alone or

whereas 6 of the 10 partially thyroidectomized animals and 16 of the 18 intact controls had hepatomas. Reduction in the incidence of liver tumours induced by AAF in rats treated with thiouracil had been reported by Paschkis, Cantarow and Stasney (1948) and by Leathem and Barken (1950). The Philadelphia group found that under the influence of the goitrogen the percentage of hepatomas in males dropped from 83·6 to 20·2 and in the females from 46 per cent to zero. Leatham and Barken repeated this experiment with pair-fed rats and thus eliminated the possible effect of reduced food intake. After 8 months 50 per cent of the animals receiving the carcinogen alone had liver cancers and the rest benign cystic cholangiomas. Only one of the 8 males receiving AAF and thiouracil developed a hepatoma, but 6 had cystic lesions in the liver. Paschkis, Cantarow and Stasney (1948) included in their paper an experiment in which para-dimethylaminoazobenzene was used as carcinogen. In the males thiouracil did not modify the action of the azo dye, but it lowered the incidence of liver tumours in the females from 88 to 46 per cent. There exists therefore evidence that thyroid hormone deficiency modifies the response of the liver cells against AF and AAF and, perhaps, against butter yellow. In the writer's experience the degree of thyroxine deficiency is crucial for the outcome of the experiment. Animals, the pituitary of which still contained acidophilic cells in appreciable numbers, always showed the typical liver lesions which follow the administration of the aromatic amine. Rats with pituitaries void of acidophils, i.e. animals in which only traces of thyroxine could still be present in the body, had livers free of neoplastic changes. It should be kept in mind that a severe degree of thyroxine deficiency implies lack of growth hormone. The endocrine imbalance is rather complex since adrenals and gonads too are affected by complete thyroidectomy. However, gonadectomy gives only a very slight protection against the hepatoma-inducing action of AAF. From the studies on tumour induction by AF and AAF in thyroxine deficient animals it became evident that the protection given by thyroidectomy was restricted to the liver, whereas the susceptibility of the sebaceous glands of the meatus acousticus externus was not affected and that of the retrobulbar glands was even increased.

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The skin seems to be an organ in which hormone deficiency does not greatly

modify the reaction to chemical carcinogens. Korteweg and Thomas (1939) studied the action of 3,4-benzpyrene on the skin of hypophysectomized mice. In one experiment the interval between operation and the start of painting the skin was 4 weeks, in another 5–7-months-old animals were used hypophysectomized at the age of 6 weeks. Skin tumours developed in all the control animals, papillomas appearing 44–100 days and carcinomas 93–104 days after onset of treatment. All of the 14 hypophysectomized mice still alive when the first papilloma was found on the 105th day of the experiment developed tumours of the skin; ten of them became cancerous between the 121st and 190th day. Although carcinogenesis was retarded in the hypophysectomized mice, the response of their skin to benzpyrene was qualitatively the same. Hall and Bielschowsky (unpublished results) repeated the work of Korteweg and Thomas (1939) using methylcholanthrene as carcinogen and restricting the application of this compound to 23 paintings. Our results are in excellent agreement with those of the Dutch workers. Benign and malignant tumours of the epidermis appeared after the administration of methylcholanthrene had been terminated in completely hypophysectomized mice showing the typical atrophy of the gonads and all the other morphological signs of pituitary deficiency. The neoplasms, however, developed slower than in the controls. One melanoma was found in a completely hypophysectomized female.

The histology of the reaction of muscle and connective tissue to methylcholanthrene in the hypophysectomized rat has not yet been described. The few data available indicate that once a hydrocarbon has induced a sarcoma, hypophysectomy will only retard its growth (Ball and Samuels, 1936). In the case of the liver of thyroidectomized rats the carcinogen apparently fails to change normal into neoplastic cells, i.e. the initiation of the neoplastic process is inhibited.

There is some suggestive evidence that other factors may be involved. Hypophysectomy seemingly does not affect the skin and its appendages to judge from the good condition of the fur and the rapid regrowth of clipped hairs in mice. Zeckwer (1953) noted that in the adrenalectomized rat regrowth of hair was even accelerated. Fraser and Nay (1953) observed a similar effect in ovariectomized mice, but inhibition of hair-growth towards the end of pregnancy, confirming earlier findings of the inhibitory action of elevated doses of oestrogen. In this connection recent results of Engelbreth-Holm and Jensen (1953) are relevant. The injection of growth hormone into mice failed to alter the incidence of skin tumours induced by a single application of 9,10-dimethyl, 1,2-benzanthracene. The only effect noted was a delayed appearance of neoplasms in the injected animals. From such observations and those of Korteweg and Thomas (1939) it seems that hormones have but little influence on the normal and neoplastic growth processes in the epidermis. On the other hand there is ample evidence that pituitary secretions can influence the growth of liver and of subcutaneous tissues as seen in acromegaly or after the injection of crude pituitary extracts.

III. The Role of Thyroid Hormone Deficiency in Tumourigenesis in Thyroid and Pituitary.

During the last decade benign and malignant neoplasms of the thyroid of rats and mice have been obtained by various means. The conditions under which they occur are : (1) After administration of goitrogenic substances alone or

in combination with carcinogens. (2) After administration of radio-iodine alone or in combination with goitrogens or acetlyaminofluorene. (3) After feeding an iodine deficient diet, and (4), possibly, after transplantation of thyroid grafts into the spleen.

1. *Thyroid Tumours Induced by Goitrogens in Rats.*

In the course of their systematic studies on experimental goitre Griesbach, Kennedy and Purves (1945) encountered neoplastic lesions in the thyroids of rats which had been kept for prolonged periods on regimens containing 45 per cent rape seed, the goitrogenic action of which had been discovered by Hercus and Purves (1936). After uninterrupted feeding of such diets single or multiple nodules of macroscopic size were present in the hyperplastic, hyperaemic glands, the first adenoma appearing in a rat receiving an iodine-poor rape seed ration for 8 months. The incidence of tumours rose to 80 per cent after 18 and to 100 per cent after 24 months. A supplement of iodine given to one group reduced the degree of hyperplasia in the thyroid, but had no influence on the number of thyroid tumours present in rats kept for 15 or more months on this regimen. None of the neoplastic lesions discovered in the thyroids of rats on the brassica-seed rations showed the morphological signs of malignancy, but one carcinoma of the thyroid developed in one of 10 animals receiving an iodine-poor control diet. The rats of the local colony were found to be more susceptible to the goitrogenic effect of rape seed than Wistar rats obtained from outside. Repeating this experiment with thiourea as goitrogenic agent Purves and Griesbach (1946) found 2 carcinomas in 30 rats, all of which had adenomatous thyroids. The diagnosis adenocarcinoma was based on the presence of multiple metastases in the lungs. In their next publication Purves and Griesbach (1947) amplified these findings and recorded the presence of 7 adenocarcinomas in 13 rats treated for 20 months with thiourea. Being familiar with the morphological changes occurring in the pituitary after administration of goitrogens, correctly interpreted by the New Zealand workers (Griesbach, 1941 ; Griesbach and Purves, 1945) as indicating stimulation of the thyrotrophic hormone (TSH) secreting basophils in response to thyroid hormone (TH) deficiency, Griesbach, Kennedy and Purves (1945) concluded that the thyroid tumours were the result of prolonged stimulation by TSH.

In 1944 the writer described the induction of tumours of the thyroid of rats by the simultaneous administration of a goitrogen, allylthiourea (Kennedy, 1942) and of a carcinogen, 2-acetylaminofluorene (AAF). The earliest neoplastic changes were seen in the thyroid of an animal killed on the 197th day of the experiment and one month later all rats had multiple adenomas. Two of the lesions were considered malignant because the neoplasms had invaded neighbouring structures. No adenomas were found in the control group treated with allylthiourea alone, nor have I ever observed thyroid tumours in rats with non-stimulated thyroids after treatment with AAF. However Armstrong and Bonser (1947) found two thyroid cancers and one colloid goitre in mice treated with this carcinogen and Cox, Wilson and DeEds (1947) 11 thyroid tumours in 84 rats. In continuation of his studies the writer (1945) tested the action of allylthiourea on the thyroid of rats treated previously for 25 weeks with AAF. After ten weeks of goitrogenic stimulation multiple adenomas were already present in the thyroids.

Of the 11 controls treated with allylthiourea alone for 6–14 weeks, 4 had minute adenomas, discovered by serial sectioning of the glands. Repetition of the experiment using piebald rats and reducing the administration of the carcinogen to 15 weeks, led again to the formation of multiple thyroid adenomas in most of the animals after 10 or more weeks of treatment with the goitrogen. No adenomas were found in the controls—proof that this strain was less sensitive to goitrogens than the Wistar rats used in previous experiments. In the same paper the fate of the single adenomas induced by allylthiourea in Wistar rats was described, the animals being killed 2–8 weeks after withdrawal of the goitrogen. The changes were identical with those observed by Griesbach *et al.* (1945) after the administration of physiological doses of thyroxine for 3 weeks. The tumour epithelium changed from tall cylindrical to a low cuboid type and colloid accumulated in the nodules. It is of interest that Purves and Griesbach (1946) could reproduce the thyroxine effect in a thyroid cancer and its metastases. When doses of thyroxine exceeding the daily requirement of the rat were administered, the thyroid tumours underwent the same involutionary changes which could be reversed by renewed administration of thiourea. Purves and Griesbach (1947) noted that under thyroid treatment colloid accumulated only in those benign or malignant neoplasms which showed a follicular structure.

Paschkis, Cantarow, and Stasney (1948) found in 93·6 per cent of their animals, treated with AAF and thiouracil, adenomata and in 14·8 per cent carcinomas of the thyroid. Of the rats on thiouracil alone 55 per cent developed benign thyroid tumours and only one neoplasm showed malignant changes. Hall (1948) repeated Bielschowsky's (1945) experiment giving 6 doses of AAF, a total of 15 mg., to each rat before methylthiouracil administration was started. Whether the stimulation of the thyroid by the goitrogen commenced immediately after the withdrawal of the carcinogen, or after an interval of 4–18 weeks, all pre-treated animals developed multiple adenomas which appeared earlier than the few single nodules found in the controls. Hall and Bielschowsky (1949) inquired whether such small amounts of AAF given prior to methylthiouracil administration played any role in the malignant change which can occur ultimately in glands after prolonged stimulation by a goitrogen. In the early stage of the experiment more numerous and larger adenomas were noted in the rats pre-treated with AAF, but all differences between them and the controls receiving methylthiouracil only disappeared after 18 months. Morphological signs of malignancy became recognizable in both groups at about the same time. On the other hand, adenomas induced by allylthiourea were not transformed into cancers when AAF was given subsequently to the withdrawal of the goitrogen. Doniach's (1950) findings are in agreement with Bielschowsky's (1945) and Hall's (1948) observations. In order to demonstrate that lack of thyroid hormone was the essential factor in the pathogenesis of these thyroid tumours Bielschowsky (1949) administered AAF to partially thyroidectomized rats. In the hyperplastic isthmus of these animals adenomas developed in the course of 18–37 weeks. Such lesions do not arise in 8 months in the isthmus of untreated partially thyroidectomized rats.

Thus AAF, even in small amounts, speeds up the development of adenomas of the thyroid of rats treated with goitrogens. At the same time it increases the frequency of the nodules. Large amounts of the carcinogen favour the rapid development of carcinomas.

Money and Rawson (1947) obtained only benign neoplasms of the thyroid by

the administration of thiouracil to male rats for periods up to 18 months. Similar observations were recorded by Laqueur (1949).

In a second communication Money and Rawson (1950) give a detailed, well illustrated account of the histogenesis of these adenomata. They found no differences in the morphology of the tumours obtained in rats treated with thiouracil alone or in combination with dibenzanthracene. In one animal, hemithyroidectomized after 16 months of thiouracil treatment, and kept without goitrogen for an additional 9 months, thyroid adenomas were still recognizable in the remaining lobe which in all other respects had reassumed its normal appearance.

Sellers, Hill and Lee (1953) treated groups of 70 Wistar rats of both sexes for 15 months with propylthiouracil. Of the three experimental groups one received propylthiouracil alone, the two others the goitrogen supplemented by either NaI or dried thyroid powder. The body weight of rats receiving the goitrogen alone or in combination with iodide remained stationary, but the animals provided with thyroid powder gained weight. The latter had the largest thyroids and those receiving the iodide supplement the smallest. Neoplastic thyroid lesions developed in all groups. Four metastasizing tumours were found, one in the propylthiouracil group and two or three in the rats receiving additional thyroid powder. It is obvious from the iodine content of the thyroid glands at the time of post mortem as well as from the plasma iodine concentration that the amounts of thyroxine provided by the dried thyroid powder were insufficient to abolish completely the thyroid hormone deficiency induced by the goitrogen. This is confirmed by the morphology of the pituitaries which showed obvious signs of thyroxine deficiency in all three groups. In this connection some unpublished results of Hall might be mentioned. He succeeded in suppressing the appearance of hyper- or neoplastic changes in the thyroids of rats treated with thiouracil by supplying thyroid hormone in amounts corresponding to the daily requirements, i.e. 2·5 μg. of d-l thyroxine per 100 g. body weight. The maximal degree of hyperplasia found by Sellers, Hill and Lee (1953) in rats which received propylthiouracil and thyroid powder is best explained by the state of health of these animals. In my experience a high degree of thyroxine deficiency which does prevent body growth is not necessarily optimal for the induction of hyperplasia or neoplasia in the thyroid. Clausen (1953) in a long term experiment with thiourea found, apart from the usual adenomas, marked degenerative changes combined with extensive lymphocytic infiltration of the gland, a picture which bore some resemblance to struma lymphomatosa.

bis-4-Acetamino-phenyl-selenium dihydroxide seems to be a very potent goitrogen to judge from a report of Seifter, Ehrich, Hudyma and Mueller (1946). They fed diets containing 0·05 per cent of this compound to rats and already at the 105th day of the experiment multiple adenomas were present in the thyroids. When smaller amounts were fed over periods of 1–2 years the degree of hyperplasia observed was rather mild and limited to the interior of the gland ; only one adenoma was found. Benign liver tumours appeared in these rats, suggestive of a direct carcinogenic action of the compound (Seifter, Ehrich and Hudyma, 1949).

In the foregoing the terms adenoma and carcinoma have been used. That we are dealing not with simple nodular hypertrophy but with neoplasms is obvious from the behaviour of the nodules. They persist when the stimulus which induced them is no longer active. Their morphology, however, changes when the goitrogen is withdrawn or when thyroxine is injected in adequate amounts to suppress

further stimulation by TSH. The epithelium of benign as well as of metastasizing tumours tends to assume under these conditions a shape akin to that of a resting thyroid. At the same time colloid accumulates in these structures as it does in the non-neoplastic part of the gland. These neoplasms vary considerably in size and morphology—some are minute structures comprising only a few acini, others are macroscopically visible nodules considerably larger than the thyroid of a normal rat. There exist two main types : solid and cystic ones. The solid micro- or macro-follicular adenomas stand out from the surrounding tissue by their densely packed cells with hyperchromatic nuclei ; they can contain some colloid when the rest of the gland contains none. In others the cells are arranged in the form of tubuli and in some the tumour epithelium grows in solid sheets, divided by strands of connective tissue, and forms acini only occasionally. The other type is characterized by papillomatous formations growing into cystic, colloid filled spaces. The degree of hyperaemia in these adenomas is remarkable, exceeding that of the rest of the gland. Large, blood-filled sinuses are found not only inside the nodules, but also often where they border the non-neoplastic tissue, and only a single layer of endothelium may separate the tumour tissue from the large vascular spaces. Regressive changes are not rare. Signs of recent or past haemorrhages are, perhaps, not as common as in human goitres of long standing. Thus one finds areas where haemosiderin-filled macrophages are lying in a dense connective tissue. Calcification is rarely seen. The great majority of the benign thyroid tumours have well-defined borders without possessing a true capsula. Once they have reached a certain size they compress invariably the surrounding tissue.

Most workers in this field have used the criteria of classical morbid anatomy for the diagnosis of malignancy : invasion of neighbouring structures, penetration of the capsule of the thyroid and metastases, most of which are blood-borne and therefore located in the lungs ; but spread to regional lymph nodes has also been observed. The incidence of metastases is not related to the structure of the primary tumour ; they occur with well-differentiated as well as with anaplastic growths. However, both the scirrhous cancers I have seen were invasive.

These morphological criteria of malignancy do not imply that the so-called carcinomas no longer need TSH for further growth. When transplanted they do not grow in normal animals, but they take in thyroxine-deficient rats, where they reach considerable size without killing the animal (Bielschowsky, Griesbach, Hall, Kennedy and Purves, 1949). When serially transplanted they can change into highly malignant neoplasms, which may kill normal rats within three weeks (Purves, Griesbach, Kennedy, 1951). Money and Rawson (1950), however, succeeded in transplanting into a normal recipient neoplastic tissue obtained from the thyroid of a rat which had been treated with thiouracil and dibenzanthracene. After a lag of 10 months the implant started to grow rapidly and invaded the surrounding tissue.

2. *Thyroid Tumours Induced by Goitrogens in Mice.*

At the same time as these investigations were pursued in the rat, workers in the National Cancer Institute Bethesda and elsewhere attacked the problem in mice. Dalton, Morris and Dubnik (1945) at first failed to find evidence for neoplasia in the hyperplastic thyroids of mice treated with thiourea, but later it

became obvious that no fundamental difference existed between mice and rats in their response to goitrogens. In 1948 the same authors gave an account of the morphological changes seen in the organs of female C3H mice after long-term ingestion of thiourea and thiouracil. Both compounds induced a remarkable hyperplasia of the thyroid. After approximately three months pale staining " parafollicular " cells became increasingly numerous, but obvious neoplastic lesions were absent. Thyroid tissue which had broken through the gland's capsule or, in another case, was found between trachealis muscle and the tracheal epithelium, was considered non-neoplastic and its unusual location due to pressure and not to invasiveness. After twelve months of thiouracil treatment colloid-containing follicles increased in numbers and the nuclei of the cells lining them were smaller and more basophilic. The lungs of approximately 40 per cent of animals sacrificed after 362–464 days contained nodules of thyroid-tissue, remarkably like the acini of the hyperplastic thyroid gland. When C mice were treated with thiouracil (Dalton, Morris, Striebich and Dubnik, 1950) for ten or more months nodular structures appeared in the thyroid. They were of two types, one formed by large cells with vesicular nuclei and the other by smaller, well defined elements with more basophilic nuclei. Only one pulmonary metastasis was seen in this experiment. In view of the poor cytological evidence for a neoplastic change, Morris and Green (1951) studied the behaviour of serially transplanted " hyperplastic " thyroid tissue. The recipients were mice maintained on a thiouracil-containing diet. In one line, the original graft coming from a mouse treated with AAF and the goitrogen, autonomous behaviour, i.e. ability to grow in a normal host, appeared in the third generation, another line acquired independence in the ninth. These experiments prove that in the mouse the same sequence of changes occurs as in the rat and that ultimately " autonomous " cancers result. Morris, Dalton and Green (1951) have given a detailed, beautifully illustrated account of these transplantation experiments. Starting from a small adenomatous area found in the hyperplastic gland of a C3H mouse treated with thiouracil for 18 months, they obtained two tumour lines which differed morphologically and in their iodine metabolism ; but both reached the capacity to grow in normal hosts after the seventh transfer. One tumour was characterized by a follicular structure, in the other the cells grew in tortuous cords. Gorbman (1946, 1947) administered thiourea or thiouracil to mice of several inbred strains and to some hybrids. He found nodular structures in the thyroid after treatment for 220–300 days ; they developed from the ingrowth of tiny follicles or cellular cords into colloid cysts which were most prominent during the 180–200th day of the experiment. Thyroid tissue noted inside blood vessels and in the lungs was considered to be metastatic but not neoplastic. It is of interest that, in contrast to what has been observed in the rat, all these lesions disappeared rapidly after the withdrawal of the goitrogen and that neither AAF nor benzpyrene did enhance the action of the goitrogen.

3. *Thyroid Tumours Induced by Radio-iodine in Rats.*

In a series of papers Goldberg and Chaikoff and their associates have given a description of the changes in the rat's thyroid which follow treatment with radioactive iodine. Goldberg, Chaikoff, Lindsay and Feller (1950) tested four dose-levels of I^{131}, namely 18, 300, 525 and 875 microcuries. The injected animals

were sacrificed after intervals ranging from 6 hours to 8 months. Only the long-term effects will be discussed, the two lower doses did not induce permanent changes. With the largest dose thyroid destruction was nearly complete and only in one animal glandular epithelium could be recognized 8 months later. With 525 μc. recovery from radiation damage took place to a certain extent and at the end of the experiment the thyroids of two animals were hyperplastic and resembled those after prolonged thiouracil treatment. Cells with ample eosinophilic cytoplasm and large irregular nuclei, likened to the so-called Hürthle cells of human pathology, were noted, the only atypical type of thyroid cells seen in this investigation. Money *et al.* (1953) found also " Hürthle cells " in the thyroids of rats treated with methylthiouracil, evidence that they owe their existence not necessarily to irradiation but probably to continued stimulation of the thyroid. That the presence of bizarre cells in irradiated thyroids is not interconnected with adenoma formation is evident from the findings of Maloof, Dobyns and Vickery (1952). They discovered only one adenoma in 500 rats treated with I^{131} although gross cytological abnormalities were noted in many. In a second study Goldberg and Chaikoff (1951) administered doses of 400 μc. of I^{131} and extended the period of observation to 18 months. They found in the thyroids of 2 rats multiple adenomas. Most were sharply defined, but two of the nodules blended with the surrounding tissue, were more cellular and richer in mitoses, and therefore suspect of beginning malignancy. In 1952 the same authors described the thyroid cancers present in rats treated with 400 μc. of I^{131} and sacrificed 18–24 months later. Nine of 25 animals had neoplastic lesions in the thyroid. Seven of these tumours were considered malignant because five had metastasized and the other two had invaded adjacent tissue and blood vessels. In the strain of rats used by Goldberg and Chaikoff propylthiouracil failed to induce thyroid cancers, although 24 of 125 animals developed benign adenomas.

Doniach (1950) studied the effect of radio-iodine alone or in combination with methylthiouracil and/or AAF upon the thyroid. He worked with rats of the Lister strain, a colony in which single adenomas of the thyroid occur in 25 per cent of the animals at the age of 13 or more months. Two injections of 16 μc. each were given $5\frac{1}{2}$ months apart and the rats sacrificed 12–$15\frac{1}{2}$ months after the administration of the first dose. Their thyroid glands were smaller than normally, but the majority contained two or more follicular adenomas. When the I^{131} treatment was followed by administration of methylthiouracil multiple thyroid tumours developed and in one animal most of the tissue of the gland was replaced by adenomas, one of which had metastasized to one of the adrenals. The control group treated with methylthiouracil alone had large hyperplastic thyroids containing up to three benign neoplasms. The number of animals having tumours in their thyroids was the same in the two groups treated with radio-iodine or with methylthiouracil. Exposure to I^{131} followed by goitrogenic stimulation led to the formation of neoplasms which were larger and more frequent than those induced by either agent, and on one occasion to malignant changes. Rats treated with AAF alone had normal or slightly stimulated glands and in 50 per cent of them 1–3 adenomas were present. Additional treatment with radioactive iodine did not increase tumour incidence in the thyroid. However, the simultaneous administration of the carcinogen and of methylthiouracil led to the formation of large nodular goitres and in two rats to an adenomatous replacement of most of the gland. The majority of the animals injected with I^{131} and receiving in their

drinking water AAF and methylthiouracil had moderately enlarged thyroids. In all of them adenomas were extremely numerous and one of these thyroid tumours had become malignant.

There is a striking parallelism between the results obtained in rats treated with I^{131} and methylthiouracil and those obtained by the simultaneous administration of AAF and the goitrogen in so far as prolonged stimulation intensifies the carcinogenic effect of both I^{131} and AAF.

In a more recent publication Doniach (1953) recorded the results of a second experiment in which the action of 30 μc. of I^{131} alone or in combination with methylthiouracil was investigated and compared with the effects obtained with 5 μc. and 100 μc. respectively. The duration of the experiment was 15 months and the age of the rats at start 10 weeks. Of the three dose levels of I^{131} tested 5 μc. and 30 μc. seemed equally effective as far as tumour formation was concerned. Both induced thyroid adenomas in 50 per cent of the injected animals, whereas the glands of the rats treated with 100 μc. did not contain neoplastic lesions. Still more impressive were the differences in the thyroids exposed to 5, 30 or 100 μc. when the glands were subsequently subjected to goitrogenic stimulation. All rats pretreated with 5 μc. had multiple benign adenomas of a size never reached by the neoplasms found in the thyroids of animals treated with methylthiouracil alone. In rats injected with 30 μc. and treated with the goitrogen afterwards the thyroid tumours were still larger and 5 of them obviously malignant. The thyroids of rats injected with 100 μc. failed to respond to goitrogenic stimulation and relatively few adenomas of moderate size developed.

The work of Doniach and of Goldberg and Chaikoff has established that amounts of I^{131} which damage the thyroids but still allow them to respond to stimulation by TSH can be expected to induce neoplastic lesions in this organ. The tumours observed under these experimental conditions did not differ essentially from those seen after prolonged administration of goitrogens despite the presence of many bizarre elements in the irradiated glands. The accompanying changes in the pituitaries have been well described by Goldberg and Chaikoff (1950) and by Doniach (1953), and have been interpreted as being due to impaired thyroid function.

4. *Thryoid Tumours Induced by Radio-iodine in Mice.*

Gorbman (1949) using a wide range of doses of radio-iodine failed to detect any neoplastic changes in thyroids of mice injected at the age of 6–9 weeks. Speert, Quimby and Werner (1951) injected 200 μc. of I^{131} into pregnant mice during the last three days of gestation and studied its effect on the offspring. There was evidence of thyroid damage early in life, but when the animals were 5 months old the glands had recovered and took up I^{131} in normal quantities. Four to seven months later the thyroids were found to have a fibrotic centre, and to contain hyperplastic nodules with areas showing papillary ingrowth into follicles as well as colloid-filled cysts lined by a flat epithelium. Rugh (1951*a*) established that a major proportion of radio-iodine injected into a nursing mouse is excreted with the milk and therefore affects the suckling offspring. In a second communication (1951*b*) he described the changes which occur in the thyroids of mice nursed by mothers which had been injected on the 3rd day of lactation with doses of 3–20 μc. of I^{131} per g. body weight. No tumours were found in the thyroid of the offspring 10 months later.

5. *Thyroid Tumours Due to Iodine Deficiency.*

The occurrence of nodular goitres in rat colonies kept in localities where goitre is endemic was first noted in Switzerland by Langhans and Wegelin (1919), and Wegelin (1927) recorded later the presence of malignant lesions in such enlarged thyroids. A similar observation was recently made in New Zealand, another country with endemic goitre. In spring 1951 enlarged, hyperaemic thyroids were seen in a fair number of experimental and of untreated rats of our colony, a phenomenon not observed by the writer in a large number of autopsies carried our during the previous three years. The fact that all cases of " spontaneous " thyroid enlargment were found at the same time and the disappearance of these goitres after providing the animals with additional iodide in the drinking water suggested iodine deficiency as the aetiological factor (Bielschowsky, 1953). Only the tumours found in untreated rats will be considered. Although the tumours seen during the active phase of the disease were of fair size, only one was recognizable at naked-eye inspection. Some were macrofollicular, others tubular and some were undifferentiated. All but one were well-defined benign neoplasms, the exception being one of the less differentiated, solid tumours which seemed to invade the surrounding hyperplastic thyroid tissue. The neoplastic lesions discovered in the resting glands of two aged animals 3–7 months after supplementary iodide had been provided, were rich in colloid and resembled closely the cyst adenomas seen in thyroids of rats after cessation of goitrogen treatment. Axelrad and Leblond (1953) studied the induction of thyroid tumours in rats fed a low iodine diet alone or with the addition of AAF. After one year a high percentage of animals in both groups were found to have thyroid tumours, some of which showed histological evidence of malignancy. These observations are in line with those of Hellwig (1935), one of the earliest workers to obtain thyroid tumours by means of an iodine deficient diet.

6. *Thyroid Tumours in Intrasplenic Grafts.*

According to some European and South American workers intrasplenic transplantation of thyroid tissue after complete removal of the gland from the trachea leads to a hyperplasia of the graft (Gabe and Arvy, 1947). Brachetto-Brian and Grinberg (1951) as well as Lacour, Oberling and Guérin (1951) observed nodular hyperplasia in intrasplenic thyroid grafts 14–15 months after the operation, and Cordier, Craps and Martin (1951) recorded the appearance of one trabecular adenoma, the only observation of this kind. Neither Bondy (1951) nor Rupp (1952) found evidence for thyroid hormone deficiency in rats bearing thyroid grafts in their spleen, nor did they find histological signs of increased activity in the transplanted thyroid epithelium. Thus the Biskind technique, so successful in the case of ovary and testis, hardly ever leads to the development of thyroid tumours, a result to be expected because this method more often than not fails to induce a thyroid hormone deficiency. However, Pasqualini and Mancini (1951) discovered in the pituitary of rats bearing thyroid grafts in the spleen changes characteristic of partial thyroidectomy.

7. *Functional Activity of Thyroid Tumours.*

Doniach (1950) was the first to obtain evidence of functional activity in an experimental thyroid tumour. He found that 12 days after cessation of treatment

with AAF and methylthiouracil a follicular adenoma took up I^{131}, although to a lesser extent than the surrounding tissue. Purves, Griesbach and Kennedy (1951) worked with a transplantable thyroid tumour growing in rats which received 0·01 per cent methylthiouracil in the drinking water. They found that the graft could concentrate I^{131} and assimilate it into organic form, despite the presence of the goitrogen. From the study of the pituitaries of thyroidectomized rats bearing such transplants the conclusion was drawn that the graft secreted thyroid hormone. Well granulated acidophils, absent from the pituitary of rats lacking TH, reappeared in the adenohypophysis and became rather numerous ; the counts of basophilic cells gave values below those characteristic for complete thyroidectomy. These results are representative for transplanted tumours which can grow only in thyroxine deficient animals. An anaplastic thyroid tumour which could be propagated in normal rats was not functionally active. Wollman, Morris and Green (1951) published data on the I^{131} uptake of four different lines of transplantable thyroid tumours and of the thyroid glands of their hosts. One of the grafts took up very small and two moderate amounts, but the fourth was functionally so active that it depressed the normal activity of the host's thyroid. This transplant incorporated more I^{131} into thyroxin than the thyroid of the tumour-bearing mouse. Wollman, Scow and Morris (1953) furnished additional data on the iodine metabolism of 6 tumour lines three of which depended still on continued thyrotrophic stimulation for their growth. They found that the grafts of the three dependent tumours could perform both functions of the normal thyroid to various extents ; they could concentrate I^{131} and bind it in organic form. Even in the functional most active tumour graft the latter function was more impaired than the former. Of the three independent lines one had lost all functional activity, but the other two could still incorporate iodide into organic binding. Attempts to alter the iodine metabolism of one independent tumour by treating the host with goitrogens failed. In contrast to what has been seen in some human cancers or in experimental tumours of the rat, the incorporation of I^{131} into an organic molecule was nearly completely blocked by propylthiouracil and the capacity of the tumour to concentrate iodine remained unchanged. Of great interest are the investigations of Money, Fitzgerald, Godwin and Rawson (1953), who studied I^{131} uptake by the thyroids of rats kept for up to 700 days on a thiouracil containing diet. Twenty-four hours before autopsy the animals were injected intraperitoneally with 5 μc. of I^{131}. Radio-autographs of paraffin sections of the adenomatous goitres revealed the presence of areas rich in I^{131}. Usually in rats treated with a goitrogen the hyperplastic thyroid tissue takes up less iodine than normal thyroid tissue, and most of it is found in the inorganic fraction. The fact that the I^{131} was not lost during the preparation of the sections suggested that iodine had been incorporated into an organic molecule. It was located in atypical follicles and in adenomas, whereas the non-neoplastic tissue seemed free of radio-activity. Thus a new type of cell had apparently come into existence which could accumulate and bind in organic form I^{131} in the presence of thiouracil. Three possibilities exist : either the new tissue was no longer affected by concentrations of goitrogen which blocked thyroxine synthesis in non-neoplastic follicles or it had acquired the ability to incorporate iodine into organic molecules by new pathways. In this case the end-product of the synthetic process would probably be different from that formed normally. The third possibility would be a reduced ability of the neoplastic tissue to concentrate the goitrogen, whereas the hyper-

plastic follicles still performed this function. As shown by Schulman and Keating (1950) and by Schulman (1950) the thyroid can achieve a thirtyfold concentration of thiourea.

The work on the iodine metabolism of experimental thyroid tumours and their grafts has furnished results which are in good agreement with those obtained in human cancers of the thyroid. Rawson, Skanse, Marinelli and Fluharty (1949) noted that thiouracil interfered less with the uptake of iodine by functional carcinomas than by normal thyroid tissue. In man as in rodents the well differentiated tumours are prone to concentrate the isotope (Dobyns and Lennon, 1948; Fitzgerald and Foote, 1949). In most instances the amounts trapped are far below those taken up by the normal thyroid. However, occasionally considerable quantities may be retained, as for instance by the rare thyroid cancers which produce signs of hyperthyroidism (Seidlin, Marinelli and Oshry, 1946).

Many non-functional metastasizing tumours will take up radio-iodine after total thyroidectomy or in response to injections of TSH or to the administration of a goitrogen. After surgical removal of the thyroid Rall, Miller, Foster, Peacock and Rawson (1951) noted an increased uptake by the metastases in 21 of 30 patients under the influence of thiouracil. Of great theoretical interest is their observation that some of these tumours acquired rather abruptly the capacity of collecting I^{131}, whereas others did so only gradually. Since a follicular structure seems to be the pre-requisite for the trapping of iodine these observations imply a change of the neoplasm towards differentiation.

8. *Pituitary Tumours.*

Enlarged or obviously neoplastic pituitaries have been observed by many workers studying the effects of long continued TH deficiency in rats or mice. It took rather a long time to establish the significance of these lesions because several obstacles militated against their proper assessment. In the rat they occur most frequently at an age when " spontaneous " pituitary tumours make their appearance. In addition they may even have some resemblance to the " spontaneous " tumours. Better understanding of the normal histology of the rat's pituitary and the introduction of new staining techniques have helped to trace their development from the TSH producing basophils of the adenohypophysis. The best evidence, however, comes from the study of the pituitary tumours which can be readily induced in several strains of mice by I^{131} given in thyroid destroying amounts. They were discovered by Gorbman, and Furth and his associates established their nature. Gorbman (1949) found that doses of 80–300 μc. of radio-iodine led to a progressive enlargment of the pituitaries, well recognizable already 150 days after the injection. Large tumours were present 100 or more days later, their size being related to the dose given, the largest tumours appearing in the group treated with 300 μc. Mice kept on a thiouracil containing diet for 400 days had enlarged pituitaries, but no neoplasms in the adenohypophysis. Whereas 200 μc. I^{131} were needed for near complete destruction of thyroids of mice on a well balanced diet, 30 μc. achieved nearly the same effect in animals previously fed a low iodine ration; but such mice did not develop pituitary tumours (Gorbman, 1951). Since implantation of thyroid tissue and to a lesser degree administration of thyroxine prevented pituitary enlargement as well as tumour formation in mice receiving effective amounts of I^{131}, Gorbman (1952) came to the conclusion

that ionizing radiations in combination with TH deficiency were the essential factors for the development of these neoplasms. The failure of small but thyroid-destroying doses of I^{131} to induce neoplastic growth in the pituitary was confirmed by Gorbman and Edelman (1952). The combination of whole body irradiation (545 r) and of radio-thyroidectomy by 30 μc. I^{131} was found to be effective, whereas each agent alone was not carcinogenic for the pituitary. It might be pointed out that the tumours of the adenohypophysis developing after exposure to I^{131} are not the only type which can be induced in the pituitary by radiations. Another entirely different type, has been found by Furth and his collaborators (Furth, Gadsden and Upton, 1953; Upton and Furth, 1953), in mice exposed to atomic detonations. Gorbman's results amplify observations of Goldberg and Chaikoff (1951). Having failed to induce pituitary tumours in rats with amounts of I^{131} up to 875 μc., they treated mice with 600 μc. Only marked hyperplasia of basophilic cells in the anterior lobe resulted; these cells appeared hypertrophic and showed increased mitotic activity. A supplement of desiccated thyroid prevented these changes.

From the experiments of Speert *et al* (1951) and of Rugh (1951) in which mice were exposed to I^{131} three days prior to or after birth it is evident that total thyroid destruction is not essential for the subsequent development of pituitary tumours. These workers noted a considerable degree of anatomical and functional recovery of the thyroids, and although these glands were far from normal they must have secreted some TH to judge from the near normal weight reached by these mice. Nevertheless, a considerable number of them developed pituitary tumours 10–12 months after exposure to I^{131}. Recently Silberberg and Silberberg (1953) published data on the influence of genetic factors and on the enhancing effect of a high fat diet on the development of radio-iodine induced pituitary tumours.

Furth and Burnett (1951) transplanted pituitary tumours which had developed in mice treated with I^{131} and found that these implants would not grow in normal mice, but in animals subjected previously to radio-thyroidectomy. Later Furth, Gadsden and Burnett (1952) obtained by serial transplantation tumour lines the growth of which was not any more dependent on the absence of TH. These autonomous grafts grew more rapidly than the dependent transplants, metastasized into lymph nodes and, what was most important, the presence of an intact thyroid in the host allowed the recognition of their secretory activity. Grafts of microscopic size already stimulated the thyroid of the host. When they enlarged they induced nodular goitres in due course. Assays of the hormone content (Furth, Burnett and Gadsden 1953), performed in Furth's own and three other laboratories, confirmed the high TSH content of the transplanted pituitary neoplasms. Of special interest were the assays of Evelyn Anderson, which indicated that no other hormones were present in significant amounts. Additional evidence for the TSH secretion of the transplants was furnished by the study of the iodine metabolism of grafted animals in which a marked tendency for iodine retention was noted.

The presence of pituitary tumours in mice (Moore, Brackney and Bock, 1953) and in rats treated with goitrogens for prolonged periods has been frequently observed (Griesbach, Kennedy and Purves, 1945; Seifter, Ehrich and Hudyma, 1949; Sellers *et al.*, 1953; Purves and Griesbach, 1951) and by Doniach (1953) in two rats treated with 100 μc. I^{131}. Only in the two last-mentioned publications these tumours are referred to as basophil adenomas, whereas most other authors classify

them as chromophobic. Recently the writer (1953) described the occurrence of basophil adenomas in conjunction with nodular goitres in rats suffering from chronic iodine deficiency. A similar observation was recorded by Fischer (1926) who found in two aged rats neoplasms in thyroid as well as in pituitary. Her animals came from Wegelin's colony, in which goitre was endemic. The cells forming these basophil adenomas resembled strongly normal thyrotrophs in appearance and in their staining qualities. These cytological findings together with the fact that of all pituitary dependent organs only the thyroid was stimulated, was considered evidence of their TSH secreting activity. Since this hormone is formed by a special type of basophil, the thyrotroph of Purves and Griesbach (1951*a*, 1951*b*), these neoplasms were classified as basophilic adenomas, although in some " basophilia " was not a prominent feature. Apparently as long as the animals are deficient in TH these tumours do not store TSH, and only a few of their cells stain basophilic or with the PAS or Gomori's fuchsine-aldehyde reagent, a behaviour similar to that of the non-neoplastic thyrotrophs. Therefore it is debatable whether the name basophil adenoma is the most appropriate, the term TSH-secreting adenoma would be more precise. To call them chromophobic adenomas is misleading. A degranulated thyrotroph or β cell to use Romeis's (1940) classification ought to be distinguished from a chromophobe or γ cell.

To summarize : Thyroid hormone deficiency is the one factor common to all observations reviewed in this section. Whether this deficiency is due to an insufficient iodine content of the diet, or is induced by goitrogens through interference with the enzymatic mechanism by which the thyroid synthesizes TH, or to damage or destruction of the gland by ionizing radiations, the result is always the same. The pituitary reacts to the lowered thyroxine level with an increase of those basophils which secrete TSH, a process akin to compensatory hypertrophy. In the rat it takes a long time before this process leads to irreversible changes in the adeno-hypophysis and a tumour results. In the mouse radio-iodine, perhaps by a direct radiation effect on the pituitary, speeds up adenoma formation. The thyroid reacts to thyrotrophic stimulation with hyperplasia, which is first diffuse, then becomes nodular and finally neoplastic lesions appear which can show the morphological signs of malignancy. This sequence of events can be more easily demonstrated in the rat than in the mouse. When the life of the experimental thyroid tumours and the period of stimulation are prolonged by serial transplantation, carcinomas result which grow in normal animals, i.e. they become independent of the stimulus responsible for their initiation and development. The same phenomenon has been observed with the TSH secreting tumours of the mouse pituitary. Tumourigenesis in the rat's thyroid can be speeded up by chemical carcinogens and by ionizing radiations. As Doniach (1953) has pointed out, the danger of tumour development is greatest at a certain dose level, namely with amounts of I^{131} which do not abolish the ability of the gland to react to TSH. Of importance is that all the neoplastic lesions, be it in pituitary or thyroid, can be prevented by supplying the animals with adequate amounts of TH.

9. *Cancers of the Human Thyroid.*

It is outside the scope of this contribution to review the vast literature on neoplastic lesions of the human thyroid. The conception of hyperplasia as first step to tumour formation in this organ seems to be generally accepted. Taylor

(1953) tracing the evolution of nodular goitre writes: "This series begins with a simple diffuse enlargement of the gland in a young patient and ends with the large multinodular goitre showing evidence of haemorrhage and calcification in the older individual". Admittedly regression in human nodular goitre is a more frequent event than in the rat, and such regressions occur perhaps more often at a time when the process is still reversible, i.e. in the stage of nodular hyperplasia. Nevertheless, a certain number of nodules arising in hyperplastic glands become neoplastic and sometimes malignant. Wegelin (1928) states: "Where goitre is endemic, malignant tumours of the thyroid occur in greater numbers". Statistical data (Wynder, 1952) show a remarkable decline in the death-rate from cancer of the thyroid since the introduction of iodized salt into the canton of Zurich in 1923; evidence for the etiological significance of iodine—and in consequence thyroid hormone deficiency in the causation of cancers of the thyroid. Ivy (1947) found in 2000 autopsies of adult dogs in Chicago an incidence of 89 per cent of goitres and of 1·6 per cent of metastasizing thyroid tumours before 1925. Two years later, after the introduction of iodized salt, both lesions had disappeared. Thus there exists a gratifying agreement between experimental and clinical findings. Still more satisfactory is that the type of thyroid cancer associated with endemic goitre seems preventable by a correction of the hormonal imbalance. However, as far as the human pituitary is concerned, there is hardly any evidence for tumour formation as a sequence to chronic thyroid hormone deficiency. From the foregoing it should not be assumed that the problem of thyroid cancer is a closed chapter. Especially, thyroid cancer in children and adolescents poses unsolved problems. Duffy and Fitzgerald (1950) found in the histories of 28 juvenile patients 10 in which the thymus had been irradiated between the 4th and 16th month of life. Whereas it is understandable why these neoplasms became manifest at the time of puberty, a period of increased growth rate of the gland, the etiological relationship between irradiation of the thymus and neoplasia in the thyroid is obscure. In a similar series of Warren, Alvizouri and Colcock (1953) neither irradiation of the thymus nor iodine deficiency played a significant etiological role. There is another type of carcinoma of the thyroid in which a history of previous goitre is frequently missing: the highly malignant anaplastic tumours found in elderly patients (Crile, 1953).

The question how often malignant changes occur in human nodular goitre has been answered differently and has been ably discussed by Cope (1952). Some surgeons are convinced of the great potential danger inherent in multinodular goitres and still more in the so-called solitary adenomas (Cole, Slaughter and Rossiter, 1945; Lahey, Hare and Salzman, 1950; Lahey and Hare, 1951). How erroneous the clinical impression of a solitary nodule can be has been pointed out by Hermanson, Gargill and Lesses (1952), who found nearly as many malignant lesions in multi- as in uninodular goitres. In any case statistics based on surgical material have not been substantiated by post mortem findings. Hazard and Kaufman (1952) found in 408 consecutive autopsies of adults in a so-called goitre area (Cleveland) 213 normal glands and 195 containing one or more nodules. In the goitrous glands they discovered one papillary adenoma, two papillary carcinomas and one non-encapsulated sclerozing tumour, none of which had produced clinical symptoms. It seems therefore that progress to malignancy is as rare an event in human nodular goitre as in that of the rat. When it happens it does not constitute necessarily a danger to life. Survival times of decades in untreated

cases of papillary carcinoma are not exceptional even when the first symptoms were enlarged lymph nodes—sign of regional metastasis.

10. *Experimental Thyroid Tumours, an Example of Responsive Tumours.*

Whether the experimental thyroid tumours of the rat pass through a stage where they can regress completely is not known, but most of them remain dependent on hormonal stimulation during the lifetime of the host. Even when they have become cancerous and have spread to distant sites, they can still respond to variations in the blood level of TSH. Foulds (1951) distinguishes two types of tumours, responsive and non-responsive. Few pathologists or clinicians will offer serious objections to Fould's scheme of progression of tumours, the end-point of which is the unresponsive state. The writer is in complete agreement with the statement that " progression may be halted at any stage and does not always reach its end-point within the lifetime of the host". It should, however, not be forgotten that serial transplantation by means of which an experimental tumour might reach the final stage gives the latter the advantage of immortality. In the case of the thyroid tumours two hormones, TSH and TH decide the issue. Such a clearly discernible situation is unfortunately rare. There exist neoplasms where the problem is far more complicated and where two questions have to be asked : are they responsive ? and : to what do they respond ? The literature of the last years contains examples of cancers of the breast reacting first favourably to ovariectomy. Then they recur to regress perhaps under the influence of testosterone. They resume growth after a while to be arrested by adrenalectomy or hypophysectomy. Before attempts were made to control carcinomas of the breast by these means one would have considered all widely metastasizing cancers to have reached the end-point. That may have been true for many, but certainly not for all. These remarks do not imply any criticism of Foulds' scheme and of the concept of progression as an intrinsic property of the tumour cell. They are intended to point out how difficult it can be to be sure that the end-point has been reached.

In a recent review Furth (1953) referring to the experimental tumours of thyroid and pituitary writes : " Most current theories regard the basic alterations in cancer as residing not in the host but in the neoplastic cell ", and continues " in this review tumours will be surveyed which find their origin in alteration of the host ". Cancers of known etiology like, for instance, those induced by a chemical agent, such as benzpyrene, are growth-responses of a susceptible tissue to a chemical carcinogen. In the writer's opinion such neoplasms do not differ fundamentally from those resulting from continued stimulation by the secretions of an endocrine gland. What differs is the origin of the agents and perhaps their potency. Whether one studies the histogenesis of chemically-induced skin tumours or of experimental thyroid tumours, one sees the same sequence of events : first diffuse hyperplasia, followed by nodular hyperplasia and finally by frankly neoplastic growth. This in itself is highly suggestive for the similarity of the process induced by entirely different agents in two different organs.

As far as the granulosa and lutein cell tumours of the ovary of rodents are concerned, they can be obtained by various means : by gonadotrophic stimulation alone (Biskind and Biskind, 1944 ; Lipschutz, Ponce de Leon, Woywood and Gay, 1946), by the combination of an exogenous carcinogenic agent and hormonal

stimulation (Bielschowsky and Hall, 1951) and, possibly, by a carcinogen alone (Marchant, Orr and Woodhouse, 1954). Even should it be proved that the granulosa cell tumours induced by 9 : 10 dimethyl-1 : 2-benzanthracene need for their development an elevated gonadotrophin level, there would only be a quantitative difference between them and, for instance, a methylcholanthrene-induced sarcoma. In the latter case normal amounts of pituitary secretions allow a neoplastic growth response to the carcinogen, but these sarcomas do not develop in the hypophysectomized rat (Moon *et al.*, 1952). Thus for the development of some chemically-induced neoplasms normal amounts of pituitary secretions are sufficient, some need more, and for tumourigenesis in the skin they do not appear to be necessary at all. On the other hand a fair number of neoplasms can be obtained by hormonal stimulation alone. It seems arbitrary to divide neoplasms into two categories—those induced by exogenous and other sinduced by endogenous agents. It has been argued that the experimental thyroid, pituitary and ovarian tumours differ from chemically-induced cancers because they depend for so long on continued stimulation. However, the same is the case with many neoplasms due to exogenous agents, such as the tar tumours of the rabbit's ear and the neurofibromas which appear in rats after feeding of ergot-containing diets (Nelson, Fitzhugh, Morris and Calvery, 1942). I see, therefore, no fundamental difference between a neoplastic growth response to a hormone and one to an exogenous carcinogen.

It has been pointed out before that certain thyroid tumours which were anaplastic and non-functional can become functionally active under the influence of thyrotrophic hormone. A similar observation was reported by Hooker, Pfeiffer and Strong (1947); Hooker (1948). A malignant non-functional interstitial cell tumour of a mouse changed under the influence of equine gonadotrophin to an androgene-secreting neoplasm, and although the tumour grew rapidly the primitive Leydig cells assumed the appearance of mature Leydig cells. Thus in responsive tumours growth and differentiation need not oppose each other.

IV. The Retrogression of Tumours.

On February 27, 1900, Pearce Gould, of the Middlesex Hospital, London, demonstrated patients suffering from cancer of the breast showing signs of focal or systemic retrogression. He wanted to call attention to one point: "the fact of repair in cancer". "A recognition of this fact will not affect our views of the true nature of cancer, but it will act as a constant stimulus to us to find out some method of treatment, some therapeutic agent, for this disease which will lead to this repair."

Some aspects of retrogression of tumours with special emphasis on the morphology of hormone-induced regression will be discussed in the last part of this contribution.

1. *Spontaneous Regressions.*

It is not my intention to review all recently recorded cases of spontaneous retrogression of cancer in man. Three well documented examples ought to suffice to convince the sceptic of the reality of the phenomenon.

Dunphy (1950) recorded a case of a post-menopausal woman, 54 years of age, with a tumour in the lower abdomen. Laparotomy revealed the presence of multiple tumour nodules in the omentum and of a mass which had involved uterus

and adnexae and was adherent to loops of small intestine. The tumour was considered inoperable and the abdomen was closed after a biopsy had been taken. The histological diagnosis was small-cell carcinoma. After two years the patient had improved. Eight and a half years after the operation a mass appeared in the right groin which grew slowly for one year when the patient re-entered the hospital. The original tumour in the pelvis had disappeared and the uterus seemed to be normal; roentgenograms of the lung were highly suggestive for miliary metastases. The mass in the groin, encapsulated lymph nodes, were surgically removed and found to be full of malignant growth of a structure similar to that of the biopsy. Two years later—in the interval the patient had become a diabetic—a small lump was present on the right side of the neck which had developed during the preceding twelve months. This was found to be a benign lipoma. Thus thirteen years after an inoperable carcinoma had been found and four years after removal of metastatic lymph nodes the patient was alive and without signs of malignant disease. Sumner (1953) described a case of spontaneous regression of a malignant melanoma in a young woman. Three and a half years before the patient sought medical advice a black mole above the internal malleolus of the left leg had become infected and disappeared. During a following pregnancy a lump appeared in the left groin which was not treated. Three years later, when the patient was again pregnant for six months, nodules were present in the right breast, in the left arm near the shoulder, and several smaller ones in the subcutaneous tissue of the abdominal wall and the back. On operation the tumours of the right breast, left arm and left femoral region were found to be partly solid, partly cystic, deeply pigmented structures. The histological diagnosis was malignant metastasizing melanoma. Although the prognosis appeared hopeless, an attempt was made to remove the subcutaneous nodules also. Astonishingly enough, all the incisions healed although the tumours were torn during the operation. Two months later the patient delivered a normal child. Eight months post-partum she returned with a recurrence in the femoral region which was excised, as was another found in the right supraclavicular region seven months later. From then on no further metastases appeared and the patient seemed free of neoplastic disease four years after the first operation. Apparently this not very malignant melanoma of the left leg had produced metastases to the groin during the first pregnancy; then the process became stationary until, during the next pregnancy, multiple secondaries developed. Since the surgeon disclaims complete removal of all malignant tissue the melanoma which progressed twice during pregnancy must have undergone systemic retrogression. The third example is a case of sarcoma in a male baby (Penner, 1953). The tumour, discovered when the child was two months old, measured 5 cm. and had produced a defect in the left femur. The histological diagnosis of a biopsy specimen, confirmed by F. W. Stewart and F. W. Foote, was sarcoma. No treatment was given. When the child was traced five years later there was no evidence of neoplastic disease and the mother stated that at the age of nine months the tumour had disappeared. Of interest is that at the time the sarcoma of the leg was discovered a swelling was also present in the left sterno-mastoid muscle. When the child was re-examined at the age of five and a half years this area was atrophic.

These observations illustrate that retrogression of cancers with an extremely bad prognosis can occur at any time of life. There is no hint as to the factors responsible in two of them. Only in the case of the melanoma the clinical course

suggests a hormonal influence. Whereas such cases are extremely rare departures from the usual course of malignant disease, there exist uncommon neoplastic lesions, such as papillomatosis of the larynx in children in which retrogression is the rule. Although generally of a self-limited character, regressing at the time of puberty, the condition has a high rate of recurrence and is potentially malignant. Walsh and Beamer (1950) have described two children in which the disease progressed to epidermoid carcinoma, and mention a third case. In contrast to the papillomas of the adult which are more often than not single, in children they are multiple and can spread into the trachea. Why, as reported by Cunning (1950), the papillomatosis of the upper respiratory tract in children has become increasingly rare is unknown. Attempts to bring on puberty by injection of testosterone failed to affect the papillomatosis in one case, but Broyles (1940) reports five remarkable regressions in children of both sexes under local treatment with oestrogen. Zalin (1948) had two successes with this therapy, whereas Gorrell (1952) was unable to influence the growth. That endocrine factors can influence the course of papillomatosis is clearly demonstrated by a case recorded by Holinger, Johnston and Anison (1950). The patient, a young woman, had undergone sixty-three endoscopic procedures for removal of recurrent laryngeal papillomas in thirteen years. Over this period she had three pregnancies and during each the papillomas disappeared to recur with the onset of menstruation. Whatever the etiology of the condition, there can be no doubt that hormonal factors have some influence on the regression or progression of papillomatosis of the larynx. In a way this condition shows an opposite trend to that of juvenile melanoma—it has greater growth potentialities prior to puberty. Facts like these should make one beware of generalizations. The question whether tumours are more malignant in the young than in the old cannot be answered, not even when cancers of the same organ are considered. For instance, inflammatory cancer of the breast is an extremely rare condition in women beyond the age of sixty, but when it occurs in the male patient it is found in the seventh or eight decade of life (Treves, 1953).

2. *Morphology of Regression.*

Retrogressions induced by hormonal treatment have become an accepted fact (Nathanson, 1951, 1952). Studying the literature dealing with this phenomenon one is impressed by the paucity of adequate descriptions of the morphological changes occurring in tumours regressing under the influence of hormones. An attempt has been made to collect the existent data in the hope that others might feel inclined to fill the obvious gaps. The writer feels that in this way worthwhile information could be gained which might help in our understanding of the action of hormones on malignant growth. Based on nearly a hundred years of practical experience pathologists have learned to recognize the morphological signs which are the mark of cancer. When a tumour has invaded neighbouring tissue or has broken into blood-vessels and spread to distant parts a neoplasm is considered malignant. In the overwhelming majority of cases tumours having these characteristics kill the bearer earlier or later. Thus the conceptions of clinical and pathological malignancy are not at variance except for the relatively few types of neoplasms, which are less dangerous than the unexperienced would assume from their morphology.

(a) *Conditioned Growths.*

One of the best examples of experimentally-induced tumours, which look malignant under the microscope but biologically are not, are the tar-induced skin tumours of the rabbit. Studying these neoplasms Rous and Kidd (1939) encountered a great variety of growths widely different in their morphology but uniform in their behaviour. Some had the microscopical appearance of what they were, namely benign neoplasms, but others had become anaplastic, had invaded the deeper layers of the skin and sometimes were found inside lymphatics. Yet all, except the frillhorns, regressed once tarring was left off. The papillomas faded away and only the largest persisted because of their core, the covering epithelium reverting to an apparently normal epidermis. The so-called carcinoids, i.e. the neoplasms which looked like carcinoma, underwent differentiation to papillomas after connective tissue had walled them off. Massive necrosis of a carcinoid was seen only once. The same authors (1941) described two different processes in regressing tumours, one characterized by proliferation of the connective tissue coupled with inflammatory changes and another by atrophy of the tumour epithelium with cell loss exceeding replacement. Mackenzie and Rous (1941) have offered overwhelming evidence for the persistence of neoplastic cells in apparently normal skin after what appeared to be complete regression, another extreme situation showing the limitations of morphology. Two possibilities exist : inability of the observer's eye to pick out a few abnormal cells among the mass of normal elements or, as suggested by Rous and Kidd (1941), the neoplastic epithelium resumes the appearance of the normal. The regression of these tar tumours is due to the withdrawal of the stimulus which induced them and in the absence of which they cannot maintain themselves. Therefore it is doubtful whether the connective-tissue reaction found around regressing carcinoids is different from processes of repair seen when non-neoplastic tissue has been destroyed by a vascular accident or by necrosis. Not every conditioned growth undergoing regression provokes this reaction. In the rat prolonged administration of oestrogens leads to a tumourous enlargement of the pituitary which regresses as soon as the hormonal treatment ceases (Nelson 1944). First the cells forming these " adenomas " become smaller, the large Golgi apparatus disappears and their nuclei resemble those of the " wheel " cells of the ovary of the hypophysectomized rat. Then the centre undergoes liquefaction and finally few signs of their former existence remain.

In human pathology where the etiology of a neoplasm is rarely known, one cannot be sure whether regression of a tumour might not be due to the withdrawal of the inductive stimulus or to the elimination of a promoting factor essential for the growth of the neoplasm. Were it not for the now many times observed regression of carcinomas after administration of hormones one would have little evidence that the body disposes of agents capable of opposing malignant growth.

(b) *Malignant Growths.*

Before reviewing the literature dealing with the morphology of human cancers regressing under the influence of hormonal treatment it might be useful to dwell shortly on some observations made on spontaneously regressing cancers of man and on the findings of Foulds (1952) obtained in breast tumours of mice.

Since Virchow's days pathologists have been aware of degenerative processes

in tumours and of the fibrous tissue reactions which accompany or follow these phenomena. Handley (1909) believed that regressive changes in tumours start always centrally and affect the oldest lesions first, and he described in detail the obliteration of invaded lymphatics by fibrous tissue. From the older literature only two other papers will be quoted. Erdheim (1930) described the repair of metastatic lesions in bone in a case of metastasizing ovarian cancer which had spread into many parts of the skeleton. Already at naked-eye inspection these lesions differed from the ordinary. From a detailed study of many metastatic foci in the bones Erdheim drew the following picture : retrogression of cancellous tissue starts in foci, sometimes in the centre, occasionally in the periphery until the greater part of the mass is involved. He distinguished two types, atrophy and necrosis. In the case of the former the number of tumour units diminish, their cells become pale and small, so that the stroma predominates and finally is the only remaining element. In the latter case all elements, including cancellous trabeculae situated in the metastasis, become necrotic. Mesenchymal elements derived from the stroma of the tumour, i.e. originally from the reticulum of the bone marrow, penetrate into the necrotic area while phagocytes remove the debris. The whole area of necrosis is then replaced by scar tissue. Its fibres are delicate and loosely knit, never assuming a scirrhus character. Sometimes cancer cells situated in the tissue surrounding the bone try to re-invade it from outside. When this happens the connective tissue which has replaced a necrotic metastasis appears immune against this invasion. According to Erdheim (1930) the malignant cells advancing against the scar grow like a benign adenoma, compressing the scar tissue but never infiltrating it. Erdheim laid stress on the particular quality of this connective tissue and of the phagocytes contained in it. He expressed the belief that such scar tissue possesses a local immunity against malignant growth. This paper has been quoted *in extenso* because it is one of the few accounts known to the writer in which morphological evidence is offered suggestive of a qualitative difference between ordinary connective tissue and the stroma of tumours.

In Sampson's (1931) paper on the reaction of the peritoneum to the implantation of cancer cells of ovarian origin similar opinions are expressed. He compares the reaction of the peritoneum to implanted tumour cells with that to an inert foreign body. The cancer cells are enmeshed in fibrin which becomes organized by ingrowing fibroblasts with or without the aid of vascular endothelium. The granulation tissue can then be transformed into connective tissue encapsulating the neoplastic cells. From the following quotation it will be seen that Sampson, like Erdheim, believed in an acquired local immunity to malignant growth. " Does the peritoneum of patients with peritoneal carcinomatosis actually develop a relative immunity to the implantation of cancer on its surface ? I believe that this occurs in some instances."

The hypothesis of local or constitutional immunity, still expressed in the 1940 edition of Ewing's famous work, figures rarely in modern writing, but the conception of the important role of the stroma in tumour regression is very much alive, as will be seen later.

Lastly, some unusual morphological findings from Sumner's (1953) aforementioned case of regressing melanoma deserve a short description. Of special interest are the changes in one of the lymph nodes removed from the groin. Here lymphatic tissue was only present in the periphery of the node. The centre

consisted of dense hyaline fibrous tissue with an area of calcification. In one region the connective tissue was more loosely arranged and contained malignant melanoma cells in different stages of degeneration together with plasma cells, lymphocytes and polymorphs in moderate numbers. The blood vessels had greatly thickened fibrotic walls and a narrow lumen. On the basis of these findings the pathologist assumed that the patient had received radiation treatment; however, this was not the case.

The histological changes occurring in mammary cancers of mice which retrogress after parturition were followed by Foulds (1952). At the height of their growth these neoplasms consist of radiating ducts filled with epithelial cells, many of them dividing. After parturition the neoplastic epithelium becomes desquamated and is apparently extruded by way of the ducts. At the same time the stroma becomes denser and finally a compact fibrous tissue, rich in collagen, compresses the duct-like structures. When these tumours resume their growth collagen disappears and a loose stroma surrounds the growing tubules. Although it is not known whether the regression is due to the withdrawal of a growth promoter or to an inhibitory agent active after parturition, it is interesting to note that in these mice the tumour epithelium as well as the stroma undergoes a sequence of changes which resemble those which can occur in cancers of the human breast.

3. *Morphology of Induced Regression.*

(a) *By oestrogens in mammary cancers.*

Few papers describe in detail the histological changes induced by oestrogens in mammary cancers. The first account was given by Koller (1944) in an addendum to the famous paper of Haddow, Watkinson and Paterson (1944). Koller's material came from a previously untreated spheroidal cell cancer of the breast of a woman of 64 years of age. She received 15 mg. of stilboestrol weekly by intramuscular route and in addition 1 mg. daily *per os*. The primary tumour as well as the axillary lymph nodes diminished in size and the ulcerated skin healed. Four biopsy specimens were taken in approximately monthly intervals, the first before treatment started and the others during the period of clinical regression. Originally the tumour contained 12·5–27·3 per cent of potentially dividing cells and the division rate was 2·3, 5·2, 5·6 and 7·5 per cent in the four areas studied. After one month the division rate did not surpass 4·6 per cent. In addition new cytological features appeared, vacuolation of nuclei, intensification in the stainability of the cytoplasm and abnormal mitoses. Degenerated tumour cells had increased from 2·5 to 12·5 per cent. The third specimen showed a very similar picture, but in the fourth a new rise in mitoses was found; many of these, however, were frankly abnormal. The number of degenerated cells reached 16·3 per cent. Koller interpreted his findings as an indication for primary damage to the nucleus and for stilboestrol-induced breakdown in the mitotic mechanism which became apparent in the fourth biopsy specimen.

Huguenin, Saracino and Gerard-Marchand (1951) described the reaction of two mammary cancers to hexoestrol. One patient, a woman 73 years old, suffered from an enormous, ulcerating and fungating cancer which had apparently not spread to the axilla. The pre-treatment biopsy revealed a carcinoma of predominantly glandular character, the tumour cell acini being lined by cuboid or cylindrical cells. In other areas, however, solid epithelial masses were found

spreading into the loose connective tissue. The patient received 20 mg. of hexoestrol for 9 days, 40 mg. for 53 and finally 60 mg. with excellent results. The next biopsy was performed after 2260 mg. of hexoestrol had been given. On the whole the picture resembled that of the first ; tumour cell acini surrounded by strands of fine fibres were still present, but the cancer cells showed definite degenerative changes. They were less well outlined, irregularities in chromatin content and shape of the nuclei more pronounced and occasionally the nuclei seemed to break up. These regressive changes were most advanced in the more isolated tumour units accompanied by intense round cell infiltration of the stroma. Four weeks later (3100 mg. hexoestrol) the tumour had become a scar with a small ulcer in the centre. A specimen taken from this area showed only granulation tissue with dilated capillaries and infiltrated by leukocytes, predominantly polymorphs. Next to the ulcer healthy epidermis covered a scar tissue rich in collagen and round cells. Six weeks later a fourth biopsy showed a further increase in the density of the collagen, perivascular agglomeration of round cells still persisted and occasionally minute calcium deposits were found in the section, but there was no evidence for the presence of tumour cells.

The second patient, a woman of 74 years, had a recurrence in the breast sixteen months after the primary growth had responded well to thermocautery and X-ray treatment. It was an adenocarcinoma differing from the one described above by growing in solid sheets, which were surrounded by a scanty connective tissue. The tumour was well differentiated in parts, but contained also more anaplastic areas with numerous mitoses and where invasion of blood vessels had taken place. This patient received 20 mg. of hexoestrol for 12 days, 40 mg. for 3 weeks and 60 mg. for 2 months. After two months of therapy the morphology of the carcinoma had changed into that of a quite atypical tumour. Where larger accumulations of cancer cells persisted remnants of pseudoacini were still recognizable ; but the cells were less well defined and their nuclei pyknotic or swollen. The stroma now formed the predominant part of the neoplasm. Instead of invasion of the connective tissue by the cancer cells mesenchymal cells seemed to advance in between the tumour epithelium, a picture resembling that of a foreign body reaction. Finally the dispersed tumour cells underwent lysis, sometimes whole groups becoming necrotic and occasionally impregnanted with calcium. A moderate inflammatory reaction accompanied these changes. When 60 mg. of hexoestrol had been given for two months only a small hard nodule remained in the area formerly occupied by the tumour and the lymph nodes were not any more palpable. A radical mastectomy was performed, but the wound healed so slowly that a new recurrence was suspected. A biopsy showed granulation tissue, but no tumour cells. It is of interest that a small squamous cell carcinoma of the face remained stationary at the time the breast cancer regressed. The French authors, although well aware of the regressive changes in nuclei and cytoplasm of the tumour cells, stress the importance of the stromal reaction. They believe that the epithelium-connective tissue relationship is shifted in favour of the latter and see in the sclerosis an active process and not a simple replacement fibrosis. Sirtori and Grattavola (1947) investigated the reaction of the connective tissue to oestrogen. From experimental findings and from their own observations in man they arrived at the following conclusions : Oestrogen stimulates the mesenchymal tissue in general, but this stimulation is more pronounced in the breast than, for instance, in the skin. As far as mammary cancers are concerned they state that the

cytological changes observed in tumour cells under treatment with oestrogen do not run parallel with clinical findings and that they do not explain the softening of the tumour, which is clinically so often the outstanding feature of retrogression. They interpret the beneficient effect of eostrogen therapy as due to a modification of the stroma around the tumour cell nests. It consists in the substitution of a sclerotic dense stroma by one which is loose, oedematous, rich in fibres and contains many histiocytes. They believe that the different response of pre- and post-menopausal women to oestrogen can be explained by the state of the tumour stroma. In the younger age group the stroma is already loose and cellular, whereas in the older women it is sclerotic. Only the latter will change under the influence of hormonal therapy. In a second well-illustrated communication Sirtori (1951) gives additional data. He describes the striking difference in the reaction of the mammary gland tissue of a young man to oestrogen and to androgen. The former induces proliferation of the connective tissue, i.e. hyperplasia and the latter sclerosis, considered to be a regressive condition. Sirtori found exactly the same changes in the stroma of mammary cancers after treatment with these two hormones and he considers them striking enough to allow a diagnosis of the agent used. Thus degenerative changes which admittedly occur in mammary cancer cells under oestrogen treatment are, in Sirtori's opinion, less important than the activation of the stroma which takes place not only in the primary tumour but also in the metastases.

Emerson, Kennedy, Graham and Nathanson (1953) have given the most comprehensive account of the changes which occur at the time of clinical regression in mammary cancers of patients treated with oestrogens. The core of their paper are observations on total mastectomy specimens from thirteen primary metastasizing tumours. Twelve of the patients were post-menopausal women and one was a man 54 years of age. His mammary cancer had regressed after orchidectomy and he was treated with stilboestrol when, eighteen months after the operation, the tumour showed renewed signs of growth. Like in the cases recorded by Huguenin, Saracino and Gerard-Marchand (1951) this publication provides histological evidence that under oestrogen treatment some mammary cancers undergo retrogressions of such an extent that one can speak of near complete disappearance of the malignant cells from the primary focus as well as from the axillary glands. At the same time the paper contains examples of discrepancies between the cardinal clinical sign of regression—softening of the tumour—and the histological evidence indicating progression. Two main alterations in regressing tumours were observed—loosening of the stroma and degenerative changes in the cancer cells. The predominant change in the connective tissue occurring during the first month of treatment consisted in a reduction of the formerly dense collagen and its replacement by a loose tissue formed by narrow strands of collagen fibres and by fibrocytes with large nuclei. Emerson and his collaborators compare this loosening of the tumour stroma with the changes in the periductal connective tissue of young women during pregnancy or in the pre-menstruum, and point out that here, too, this process is limited to the connective tissue adjacent to epithelial elements. In earlier phases of retrogression degenerating cancer cells, together with plasma cells and lymphocytes, were still present. Later on these cells disappeared, new collagen as well as elastic fibres formed, so that in the end there remained nothing but a fibrotic area formed by a sparsely cellular dense connective tissue. The same end result as in the primary tumour could be observed in

invaded lymph nodes. In the cases which responded best the lymph glands were transformed into scar tissue in which a few degenerated cancer cells remained. In regressing skin lesions the alterations in the connective tissue were less prominent, but occasionally lymphatics were found obliterated by fibrotic plugs. The degenerating tumour cells showed a swollen, pale or vacuolated cytoplasm, karyolysis or pyknosis of nuclei and fragmentation or shrinkage of cells. Comparison of the material obtained from patients with induced regression with that of 110 mastectomy specimens from breast cancer patients not previously treated revealed that the magnitude of the changes found in the former was of quite a different order. Foci of degenerating tumour cells and stromal changes of a similar character as described above were occasionally seen in the material from untreated patients, but the areas so affected were never large enough to suggest a general tendency for regression. Of interest is the observation that all but one of the regressing cancers showed a marked hyperplasia of the elastic tissue prior to treatment. This hyperplasia increased under the influence of oestrogens. Tumours of low to medium malignancy with a tendency to fibrosis and hyperplasia of elastic fibres were, in the experience of the American workers, most likely to show a favourable response to oestrogen. Which of the two major alterations, degeneration of tumour cells or connective tissue changes, occurs first they were unable to ascertain. There was no evidence that one preceded the other. They conclude that the main effect of the hormone is to strengthen a pre-existent reactive response of the connective tissue to the tumour and express the opinion " that these regressions were in part the result of an unusual hormonal stimulation of a natural occurring process of repair".

It should be taken into account that the findings just discussed come from a selected material, i.e. from cases showing unusual degrees of retrogression. This may explain why some authors were unable to discover consistent morphological changes in breast cancers which clinically improved under oestrogen treatment (Godwin and Escher, 1951 ; Dargent and Papillon, 1951).

A possible defensive role of the subepithelial connective tissue surrounding mammary ducts filled with cancer cells has been proposed by several authors, more recently by Bohle (1951). The findings of Huguenin, Sirtori and Emerson *et al.* give substance to this hypothesis and offer at least suggestive evidence that a defensive process against malignant growth can be set in motion by oestrogens in certain cases of cancer of the breast.

(b) *By oestrogens in prostatic cancers.*

Schenken, Burns and Kahle (1942) were the first to give a well illustrated account of the morphological changes occurring in prostatic cancers of patients treated for periods of 25–46 days with stilboestrol or its dipropionate ester. (The case histories are given in the paper of Kahle, Ogden and Getzoff, 1942.) The principal findings in these biopsies were marked shrinkage of nuclei to about half their former size and vacuolation of the cytoplasm in the tumour cells. Although slight cytoplasmic vacuolation is not an unusual feature of prostatic cancer cells, the degree observed after administration of synthetic oestrogens surpassed by a wide margin what could be found in untreated patients. The figures 14–17 of Schenken *et al.* (1942) paper give a clear picture of the different stages of cell degeneration, ranging from pyknosis of nuclei and ballooning of the cytoplasm to cell destruction, i.e. the formation of clear spaces having the shape of acini and

containing only nuclear fragments. Groups of healthy-looking tumour cells remained among the degenerating elements. Heckel and Kretschmer (1942) observed similar degenerative changes in the prostatic cancer of a patient treated with stilboestrol for 233 days. Fergusson and Pagel (1945) tried to assess quantitatively the reduction in the number of tumour units as well as the coincident decrease in nuclear size of neoplastic cells in five carcinomas of the prostate which responded with retrogression to oestrogen therapy. Tumour cell counts in a second biopsy specimen taken 10–30 months after commencement of treatment showed that their numbers had declined to one-third to one-sixth of the pretreatment values. In three cases the nuclear diameter was significantly smaller. To judge from the photomicrographs the stroma increased in amount and density during the period of treatment. The paper describes and depicts also the disappearance of acid phosphatase from metastatic tumour cells after 24 days of administration of dienoestrol. Continuing these investigations Fergusson and Franks (1953) noted that already one week after the onset of treatment regressive changes were recognizable in the prostatic cancer cells, vacuolation of the cytoplasm being the first sign. The affected cells increased in size, assumed the shape of signet cells and their nuclei became denser or, occasionally, vacuolated. Desquamation and rupture of the neoplastic cells were the end result of this process. At the same time the stroma became imbued with products of disintegration and converted into a loose, fibrillar, ill-defined tissue which had an affinity for basic dyes. Replacement fibrosis was sometimes seen at a later stage. In two cancers squamous metaplasia was observed, once in the primary tumour and once in a metastic nodule situated in the liver—possibly a reaction of the tumour cells to oestrogen similar to that of the normal transitional epithelium of the prostatic urethra. Although each type of prostatic cancer cell—the authors distinguishing between a clear or reticular, a dark basophilic and an anaplastic type with vesicular nucleus—showed approximately equal sensitivity to oestrogen, the dark elements were found more frequently among the surviving cancer cells, present in all specimens.

From these observations it appears that in the case of prostatic carcinoma the reaction to oestrogen is far less complex than in mammary cancers and, what facilitates the interpretation, the first changes appear after a remarkably short interval. Fergusson and Franks (1953) refer to observations of their own in which the neoplastic cells were already affected by oestrogen after less than 24 hours, a period shorter than that needed for the induction of oestrus in a gonadectomized rodent. These findings are, in the writer's opinion, highly suggestive for a direct effect of the hormone on the neoplastic cell.

Quite a different type of regression was seen by Franks (1953) in a case of prostatic cancer. Since treatment with large doses of stilboestrol had failed to influence pain and dysuria in the 63-years-old diabetic patient, adrenalectomy was performed. No immediate clinical improvement followed the operation and stilboestrol therapy was reinstituted. Death occurred on the 40th post-operative day in consequence of an infarction in the lung. The post-mortem examination revealed the presence of a large carcinoma of the prostate which had invaded the seminal vesicles and the bladder. Metastases were found in liver, lungs and in many abdominal and mediastinal lymph nodes. Histologically the primary tumour as well as all the secondaries showed massive central necrosis, surrounded by a rim of apparently viable tumour. The two zones were separated by granu-

lation tissue. The central necrosis involved tumour cells as well as stroma, and even some of the larger blood vessels There was a gradation in the intensity of the degenerative changes, which reached the maximum in the centre. From the study of small metastatic foci in which the blood vessels were not affected Franks concluded that the primary effect had been on the tumour cells. This case provides good morphological evidence for acute systemic regression in a primary cancer and its metastases without active participation of the stroma.

The factors responsible for the destruction of this widely metastasizing neoplasm cannot be easily assessed. However, it seems possible that the large doses of stilboestrol given were of greater importance than the adrenalectomy. Some support for this contention comes from Baker's (1953) observations on two patients, the metastatic pain of which was not ameliorated by oestrogen prior to but after adrenalectomy. Whereas an increased and sometimes even fatal androgen effect after adrenalectomy is a well-established fact, no corresponding data exist so far in the case of oestrogens.

In prostatic smears from patients treated with oestrogens Peters (1950) found regularly large highly vacuolated cells, the apparently empty cytoplasm of which was in fact filled with glycogen. These epithelial elements disappeared rapidly from the smear when hormone therapy was withheld. According to Peters these glycogen-rich cells are non-neoplastic and easily distinguishable from carcinoma cells, which are shrunken, have poorly stainable nuclei and can disappear under oestrogen treatment (Peters and Frank, 1952). Papanicolaou (1949), however, mentions that not only normal but also prostatic cancer cells may show cellular and nuclear enlargement under these conditions. I have been unable to find any reference to the glycogen content of the vacuolated neoplastic cells characteristic for an oestrogen senstive prostatic carcinoma. The pictures of Peters (1950) resemble so much the balloon cells of Schenken *et al.* (1942) and of Fergusson and Franks (1953) that it seems worthwhile to investigate whether the neoplastic prostatic epithelium accumulates glycogen under the influence of oestrogen.

(c) *By progesterone in cancer of the cervix.*

Hertz, Cromer, Young and Westfall (1951) discovered that some carcinomas of the cervix of pre- and post-menopausal women regressed under progesterone treatment. Platt (1952) gave a description of the morphological changes noted in cervical smears and in biopsy specimens obtained from these patients. In cases responding favourably a change in the composition and cytology of the smear was already recognizable at the third day of progesterone administration. Blood and cellular debris diminished and normal squamous epithelial cells became more numerous. The cancer cells increased in size, their nuclei losing the compact and assuming a more granular appearance. The same trend was also seen in sections of the tumours. In a few patients cancer cells disappeared completely from the smear and post-treatment biopsies failed to reveal the presence of epidermoid carcinoma. A comparison of pre- and post-treatment tissue sections stained with Best's carmine disclosed differences in " glycogen " content of normal and neoplastic squamous epithelium. In post-treatment specimens less glycogen was found in the nuclei and hardly any in the cytoplasm ; but an accumulation of intercellular carmine-positive material was noted together with increased amounts of fluid. Platt mentions that similar effects of progesterone were seen

in one case of squamous cell carcinoma of the vulva. It seems desirable to check the shift of the Best carmine-positive material with more specific staining methods.

(d) *By other means.*

Literature on the morphology of testoterone-induced regressions of mammary cancers is practically non-existent. Adair and Hermann (1946) quote in their paper the reports of Fred Stewart on the biopsies of two of their cases which regressed under testosterone treatment. The material came from metastatic skin nodules. " Rather marked focal hydropic degeneration, mitoses still present " and " cells definitely hydropic and nuclei pyknotic. Rare mitoses still seen. The tumour shows distinct differences from the expected." I do not know of any comprehensive study which confirms or refutes Sirtori's (1951) findings of the sclerotizing action of androgens on the stroma of mammary cancers. Apparently testosterone affects more frequently metastases to the bone, and many excellent X-ray photographs testify to the far-reaching regressions they can undergo under androgen treatment. However, lesions which on radiological evidence appear completely healed can contain still considerable amounts of viable tumour, as revealed by post-mortem studies (Preston, Taylor and Crumrine, 1949).

Although excellent descriptions of clinical regressions of mammary cancer in man after orchidectomy are available (Treves, Abels, Woodard, and Farrow, 1944; Treves 1949) the corresponding morphological changes have not yet been fully described, and the same scarcity of data exists for tumours regressing after adrenalectomy and hypophysectomy.

Emerson *et al.* (1953) mention the alterations in an adenocarcinoma of the breast in a middle-aged man after orchidectomy. Three biopsies taken during the period of post-operative regression revealed degenerative changes of quite considerable extent, but already, six months after orchidectomy, foci of less differentiated carcinoma were discovered in a lymph node.

Luft and Olivecrona (1953) depict the changes which occurred in a typical gelatinous mammary cancer following hypophysectomy. Before the operation the amount of colloid was moderate and epithelial elements predominated. Four months after the operation few groups of malignant cells remained and the mucoid material was abundant. Five months later only isolated cancer cells were present, there was less mucoid material and the connective tissue had increased. Histological examination of several organs of a hypophysectomized patient who had died of malignant melanoma (Shimkin, Boldrey, Kelly, Bierman, Ortega and Naffziger, 1952) revealed some unusual features in metastatic nodules situated in liver and spleen. The two " secondaries " in the liver were found to consist of a dense collagenous scar tissue containing pigment granules, but no tumour cells. In the spleen the metastases had a hyalinized or necrotic centre and even in the periphery there was a considerable amount of fibrous tissue. Here the tumour cells appeared degenerated, some had hydropic nuclei, others were remarkably small and appeared shrunken. On the other hand, no morphological evidence for regression was found in the metastases in jejunum, lymph glands and lungs.

4. *Changes in Endocrine Organs of Patients Suffering from Malignant Disease.*

Surgical removal of the gonads, the adrenals and the pituitary has been performed with the intention of arresting the progress and spread of cancers which

could no longer be controlled by other means. Since the earliest days of this century, when British surgeons were well aware of the benefit of ovariectomy in some cases of mammary cancer, progress has been slow in the understanding why the response to the operation varied so greatly. Already in 1900 Boyd suggested "that certain ovaries, probably by pathological variation in their internal secretion, favour the growth of cancer by action either upon the growth or upon the tissues; the removal of such ovaries alone will be of benefit". Progress has come from the study of the ovaries and secondary sex organs of post-menopausal women. The old conception of a functionally inactive gland has given way to the recognition that the ovary can secrete oestrogens for considerable periods after menstruation has ceased. Examination of vaginal smears showed that in many post-menopausal women the mucosa is far from being atrophic and the same applies to the endometrium. In 1941 Smith called the attention to abnormalities in the post-menopausal ovary of women with endometrial cancer. Instead of a narrow cortex rich in collagen he found a wide cellular outer zone. These changes occurred more often in cases of cancer of the corpus uteri than in normal women. A later study by Woll, Hertig, Smith and Johnson (1948) confirmed and enlarged Smith's previous findings. The main features of the condition they described and named thecomatosis were nodular masses of plump cells, which had an enlarged nucleus and prominent nucleolus and dipped deep into the stroma. Sometimes areas of epithelial-like lipoid-containing cells were also present and were considered to be luteinized theca cells. The publications of Laffargue, Luscan and Lavernhe (1952) and of Dockerty, Lovelady and Foust (1951) contain confirmatory findings. Very similar changes in combination with medullary vascular hyperplasia were found by Sommers and Teloh (1952) in 83 per cent of patients which had died of carcinoma of the breast and in 37·6 per cent of controls. A high degree of hyperplasia occurred only once in the non-cancer group. McManus and Sommers (1952) found that women with ovaries showing thecomatosis at the time they were surgically removed had a longer survival time than patients with atrophic ovaries. In the latter group of breast cancer patients the post-castration survival was so short that the authors consider the possibility whether ovariectomy had not produced actual harm. Burt and Castleman (1953) also observed a high incidence of ovarian stromal hyperplasia in women with carcinoma of the breast.

The findings of Sommers and his associates might provide an explanation for some extraordinary post-castration regressions. Raven (1950) reported a remarkable case of metastasizing spheroidal cell carcinoma of the breast which retrogressed after ovarietomy to such an extent that two years after the operation no signs of malignant disease were detectable. In one of the ovaries of this patient the pathologist found a wide, ill-defined zone of theca cell formation.

As far as the testes are concerned they do not show specific changes in either cancer of the breast or of the prostate. In the adrenals of some of their patients with advanced mammary cancers Huggins and Dao (1953) found evidence for hyperplasia especially marked in the zona glomerulosa. Burt and Castleman (1953), too, noted that the average adrenal weight in women with cancer of the breast exceeds that of adrenals from patients without carcinoma. These authors gave the only modern description of the cytology of the adenohypophysis in malignant disease of the breast. They found an increase in the basophils and in the hypertrophic amphophils, an abnormality not specific for mammary cancer.

This important paper contains interesting observations on the effect of oestrogens on the human pituitary.

Of the morphological investigations reviewed above only those on the ovaries have contributed so far to our understanding of malignant disease. There seems to be a definite need for more information, not only on the pathology of the ovaries but specially of the pituitary, adrenals and testes.

5. *Discussion.*

The findings presented in the section on hormone-induced regression will, it is hoped, be accepted as evidence that physiological agents can influence profoundly some malignant growths. It could be argued that the therapeutic results obtained are due to the administration of pharmacological doses and therefore should not be used as evidence for a naturally occurring hormonal defence mechanism. Actually the amounts of oestrogen needed for arrest of a susceptible carcinoma of the prostate are rather small. Using the level of serum aldolase, or of acid phosphatase as indicator, Baker and Govan (1953) and Baker *et al.* (1953) found doses of 0·25–0·5 mg. effective. Similar amounts control the symptoms of the menopause without inducing hyperplasia of the endometrium. Thus they do not fall outside the physiological range. Admittedly sometimes much higher doses are needed to influence malignant growth. In systematic studies over twenty years Lipschutz (1950) and his pupils have firmly established the quantitative relationship between tumour growth-promoting steroids and those opposing it. Lipschutz (1952, 1954) has drawn attention to the danger which may result when rhythmic processes are converted into continuous ones and sees in the rhythmic release of ovarian hormone one of the means of "antitumoural auto-defence". As far as the amounts of steroid needed for tumourigenesis are concerned he found them to be much smaller than was believed previously; in fact, physiological amounts, acting unopposed, could induce neoplastic growth. With increasing knowledge of the natural history of tumours responding to hormonal action the gap between clinical and the experimental findings of the Chilean workers has narrowed.

Stewart (1952) has recently recorded his experiences in spontaneous regression of neoplasms. He considers the disappearance of *in situ* epidermoid carcinoma of the uterine cervix a distinct possibility, an opinion shared by other students of this condition (Reagan, 1952; Hoffman, Farell and Hahn, 1953). After the demonstration of the beneficial effect of progesterone on such or even more advanced lesions (Platt, 1952) one could envisage a shift of a disturbed steroid hormone balance towards predominance of the corpus luteum hormone as a possible mechanism for retrogression of these neoplasms. Slight variations in hormone level might also account for the appearance of late metastases of hormone dependent tumours.

6. *Regressions Induced by Immunological Reactions.*

Stewart's (1952) paper contains a unique observation. A patient suffering from myosarcoma of the uterus reacted to irradiation with what resembled an anaphylactic reaction and the inoperable tumour disappeared in the course of a few days. Five years later she was again treated with radium for a lesion of the cervix. Again high fever, an urticarial rash and eosinophilia appeared, proof that

the first reaction and the regression of the tumour were not due to specific sensitization against the cancer. There is little evidence that antibodies elicited by a neoplasm have ever produced enough tumour-specific antigens to induce its destruction (Hauschka, 1952). However, immunogenetical reactions may play a role in the regression of choriocarcinomas. This malignant growth of foetal origin could provoke an antigenic response when invading maternal tissue. Two facts support this hypothesis. Park and Lees (1950) on the basis of an analysis of 516 cases of choriocarcinoma conclude : " True regressions occur in a small but certainly significant proportion of cases ". Late recurrences, if they occur at all, are extremely rare as compared with carcinoma of the breast. The authors state : " Choriocarcinoma kills either within 12 months of diagnosis or not at all", a most unusual behaviour for a malignant growth. The high death-rate from choriocarcinoma recorded in the older literature was probably wrongly ascribed to the malignant properties of the tumour itself. The mortality from choriocarcinoma seems to have declined in the last decades since blood transfusion and chemotherapy have lowered the risk of death from haemorrhages and puerperal sepsis. When these complications were eliminated astonishingly high survival rates resulted. They cannot be dismissed with the assumption that the condition was overdiagnosed. For instance in a series of 22 cases described by Huber and Hörmann (1952) there were 19 survivors, in 6 of which metastases had been present.

These observations may be taken as an indication that apart from hormones other, not yet identified, bodily agents may be able to induce regression in malignant growths.

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EFFECTS OF RADIOACTIVE IODINE ON THE RAT THYROID'S FUNCTION, REGENERATION AND RESPONSE TO GOITROGENS.

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THE use of radioactive iodine (I^{131}) in tracer doses has proved extremely successful in experimental studies of the metabolism of iodine by the normal thyroid gland and together with the application of antithyroid drugs has led to considerable advances in thyroid physiology. However, there have been only few experimental investigations of thyroid function after treatment with moderately damaging doses of I^{131} given in a dosage range equivalent to that used in the treatment of Graves' disease.

We report our findings below of tests carried out, after varying intervals of time, on rats injected intraperitoneally, at the age of 3 to 4 months with 30 microcuries of carrier free NaI^{131}. The average uptake by the thyroid gland was 20 per cent of the injected dose. We have tried to find the effect of this irradiation upon thyroid weight, follicle cell height and morphology, ability to concentrate iodide and bind iodine, to regenerate after partial thyroidectomy and to respond to a powerful goitrogen. We wanted to see if any functions were damaged preferentially. We also hoped that at least one of the tests might show an easily measured consistent deviation from the normal. We proposed, if successful, to apply this test in order to find what dosage of external X-irradiation was comparable in this biological effect with 30 μC of I^{131}.

MATERIAL AND METHODS.

Rats.—(1) Black and white hooded Lister strain from a closed colony and (2) home bred albinos of mixed origin. All were fed on " Research " rat cubes and given greens twice a week.

Propylthiouracil (Williams & Co.).—The crystalline preparation was dissolved in water, 10 mg./1 ml. by the addition of drops of 40 per cent NaOH until a clear solution was obtained. Drops of 10 per cent HCl were then added until the pH was about 8·0 (B.D.H. universal indicator). Dilutions were made from this stock solution. Propylthiouracil-drinking water, 6 mg./10 ml. was made up twice weekly from fresh stock.

Histological techniques.—The thyroids were fixed, attached to the trachea, in Helly's fluid for 3 to 4 hours, washed overnight in tap water, dissected off the trachea and weighed. They were then dehydrated, cleared, embedded in wax and sectioned at 5μ, cut horizontally in a central plane so as to include both lateral lobes and the isthmus. All were stained by haemalum and eosin and by the " tripas " periodic Schiff method of Pearse (1949). Many were stained by the Feulgen technique.

Measurement of Radioactivity.—The fixed and washed thyroids still attached to the trachea were each suspended at a fixed point in a standard $1\frac{1}{2} \times \frac{3}{4}$ inches screw-capped vial and counted in the multitube gamma Geiger Müller ring counter described by Veall and Baptista (1954). The count rates given by these thyroids represented organically bound I^{131} only, since all inorganic iodide had been washed out of the tissues.

Digestion of thyroid tissue and plasma for counting (in order to estimate T/S ratios) was done in a mixture of equal parts 5 per cent NaOH and 95 per cent ethanol in an oven at 60° C. for a few hours. Each sample was digested in a total of 9 ml. fluid in a standard vial and counted as above.

Measurement of thyroid plasma (T/S) iodide ratio.—This was based on the principle and technique described by Vanderlaan and Vanderlaan (1947). Prior to injection of I^{131}, rats are pre-treated with a large dose of propylthiouracil, which prevents iodine from becoming organically bound. The measured radioactivity of the thyroid and blood is then entirely due to inorganic iodide. We injected the animals intraperitoneally with 10 mg. propylthiouracil in solution. Half an hour later each rat was injected subcutaneously with 50 μC I^{131} in 1 ml. water. The rats were killed one hour after this by bleeding from the aorta under ether anaesthesia. Thyroid tissue, removed under the anaesthetic (after bleeding), was weighed immediately on a torsion balance and put into 9 ml. digest mixture in a standard vial. The average weight removed was 17 mg. Blood, collected into an oxalated tube was centrifuged and 1 ml. plasma taken into a standard vial containing 8 ml. digest mixture. After digestion at 60° C. the radioactivity was measured. The thyroid plasma (T/S) iodide ratio of each rat was calculated from its thyroid count rate per gram of thyroid tissue divided by the count rate of 1 ml. of its plasma.

Measurement of mean follicle cell height.—H. and E. stained sections were placed on the moving stage of a microscope set up for measuring red cell diameters and the image projected on to paper at 1000 times magnification. Two long lines which crossed centrally at right angles were drawn on the paper. In any projected field the images of occasional follicles lay by chance with their largest diameters directly coincident with one of the lines on the paper. The heights of the two cells, in these particular follicles, which lay along the lines were recorded. The heights of 200 cells were measured in each thyroid gland.

EXPERIMENTS AND RESULTS.

Experiment I.

Ten out of 20 male hooded rats were injected intraperitoneally at the age of $3\frac{1}{2}$ months with 30 μC I^{131}. They and the 10 controls were killed by bleeding under ether anaesthesia 3 months later, 2 hours after an intraperitoneal injection of 10 μC I^{131}. The thyroids were fixed and washed. Measurements were made of their radioactivity (organically bound I^{131}), of their weights and of their mean follicle cell heights.

The results summarized in Table IA show that even though the previously irradiated rats were slightly heavier in body weight than the controls, their thyroids were smaller, 20·9 mg. against 26·9 mg. ($P < 0{\cdot}01$). The 2-hour uptake of I^{131} was not significantly different in the two groups. However, this "normal" uptake of the smaller irradiated glands was associated with an increased mean

follicular cell height, 7·6μ in 2000 cells against 6·6μ in the controls ($P < 0.001$). Microscopic examination showed an associated diminution in colloid in the irradiated glands (Fig. 1 and 2) whose nuclei presented a greater variation in size than the controls.

TABLE IA.—*Body Weight, Thyroid Weight, Iodine Uptake and Follicle Cell Height after I^{131}.*

Number of rats.	Treatment.	Mean body weight (g.) + S. deviation of mean.	Mean thyroid weight (mg.) + S. deviation of mean.	*Thyroid uptake of I^{131}.* Mean activity in 1000 counts/min. + S. deviation of mean (10 μC I^{131} 2 hrs. before death).	Mean thyroid follicle cell height in μ (200 cells measured in each rat).
10	Nil	303±36	26·9±3·9	5·5±1·3	6·58
10	30 μC I^{131} 3 months previously	315±23	20·9±2·9	5·7±1·2	7·64

In confirmation of this thyroid loss of weight following 30 μC I^{131} we give in Table IB data obtained from another (unpublished) experiment in which the rats were treated with colchicine 8 hours before they were killed. Male albino rats were killed in groups of 7 irradiated and 7 controls at intervals of 24, 78 and 132 days after receiving 30 μC I^{131}. The body weights of the irradiated rats killed after 24 days were higher than the controls, but the body weights of the irradiated and controls respectively, killed at 78 and at 132 days, were similar; body growth was normal in the irradiated rats in both groups. There is no statistically significant difference in the irradiated and control thyroid weights at 24 and at 78 days; but at 132 days the irradiated thyroids weighed only 18 mg. in contrast to the controls 28 mg. ($P < 0.01$).

TABLE IB.—*Body and Thyroid Weight at Varied Times after I^{131}.*

Time in days after 30 μC I^{131}	Number of rats.	Mean body weight (g.) + S. deviation of mean.	Mean thyroid weight (mg.) + S. deviation of mean.
24	Controls 7	262±19·8	23±3·6
	Irradiated 7	318±21·6	26±4·6
78	Controls 7	368±23·4	27±4·2
	Irradiated 7	364±24·1	21±2·0
132	Controls 7	387±28·7	28±6·9
	Irradiated 7	391±41·0	18±1·4

Experiment 2.

The thyroid : plasma iodide ratio was determined in 20 male hooded rats, 10 of which had received 30 μC I^{131} 3 months previously. These were siblings of the animals in Experiment I.

The results summarized in Table II show no significant difference in the T/S iodide ratio, 75 ± 13 in the irradiated and 70 ± 22 in the controls. The body weights were similar in the two groups and no different from those in Experiment I.

Experiment 3.

Having found in Experiment I that the irradiated thyroids gave a normal 2-hour uptake of I^{131}, we wondered if they would regenerate after hemithyroidec-

TABLE II.—*T/S ratio after* I^{131}.

Number of rats.	Treatment.	Mean body weight (g.) + S. deviation of mean.	Mean throid/plasma iodide ratio + S. deviation of mean.
10	Nil	308±23	70±22
10	30 μC I^{131} 3½ months previously	298±22	75±13

tomy as efficiently as controls and if such stimulated glands would show a normal uptake of I^{131}. Ten out of 20 female hooded rats were injected intraperitoneally at the age of 3½ months, with 30 μC I^{131}. Eleven weeks later, the left lobe and left half of the isthmus of the thyroid was removed at operation under ether anaesthesia from them and from the 10 irradiated controls. Nine days later all 20 rats were given an intraperitoneal injection of 10 μC I^{131} and killed after 2½ hours. The residual thyroid lobe was removed, fixed in Helly, washed, its radioactivity measured, the gland weighed and finally embedded in paraffin wax and sectioned as in Experiment I. Four additional female hooded rats, irradiated 3 months previously with 30 μC I^{131}, not hemithyroidectomized, were given 10 μC I^{131} 2½ hours before being killed and the I^{131} uptake of their thyroids measured.

The results summarized in Table III confirm the finding in Experiment I that the irradiated thyroids are smaller than those of controls 3 months after administration of 30 μC I^{131}, 7·3 mg. as against 9·4 mg. Their 2½-hour uptake of I^{131} was the same as that of the unirradiated controls. The residual lobe, 9 days after hemithyroidectomy, showed a 2½-hour uptake of I^{131} similar to that of the whole gland of the non-thyroidectomized rats.

The histological findings in the residual lobes of the hemithyroidectomized rats were interesting. In both groups the follicle cells were taller and colloid less in quantity than normal. The degree of activity varied, being more marked in the central region of the lateral lobes, many of the follicle cells there contained

EXPLANATION OF PLATES.

FIG. 1.—Thyroid of control rat. × 40.

FIG. 2.—Thyroid of rat injected 3 months previously with 30 μC I^{131} showing smaller follicles, taller epithelium and less colloid than the control. × 40.

FIG. 3.—Thyroid of control rat 9 days after hemithyroidectomy showing hypertrophied cells. × 465.

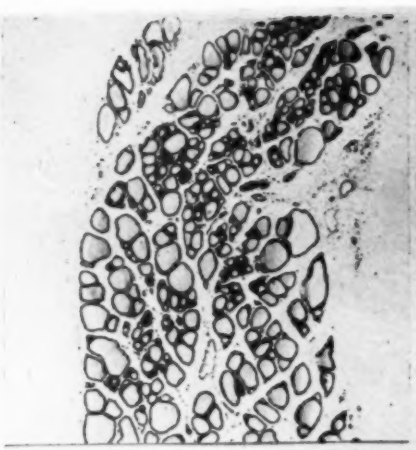
FIG. 4.—Thyroid of rat 9 days after hemithyroidectomy, injected 3 months previously with 30 μC I^{131}, showing larger cells than the non-radiated hemithyroidectomized thyroids (Fig. 3) and a greater variation in nuclear size. × 465.

FIG. 5.—Thyroid of non-irradiated rat at end of 10 days propylthiouracil showing a typical goitrogen induced hypertrophy; tall cells, markedly diminished colloid and a mitotic figure. × 465.

FIG. 6.—Thyroid of rat at end of 10 days propylthiouracil, injected 31 days previously with 30 μC I^{131}, showing a goitrogen induced hypertrophy with larger cells and a greater variation in nuclear size than in the thyroids of the non-irradiated propylthiouracil treated rats (Fig. 5). Abnormal mitoses are present. × 465.

FIG. 7.—Thyroid of rat at end of 10 days propylthiouracil, injected 91 days previously with 30 μC I^{131}, showing an even greater variation in nuclear size and scattered micronuclei. × 465.

FIG. 8.—Thyroid of rat at end of 10 days propylthiouracil, injected 130 days previously with 30 μC I^{131}, showing bizarre-shaped nuclei, marked irregularity in follicle shape and occasional cells with clumped pycnotic chromatin. × 465.



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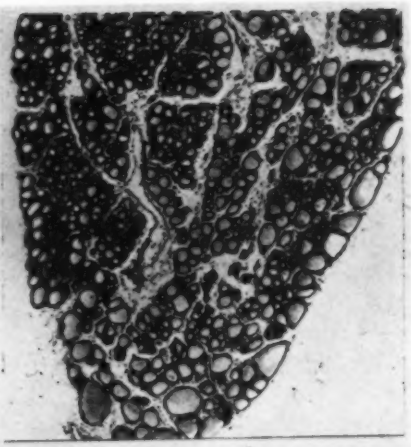

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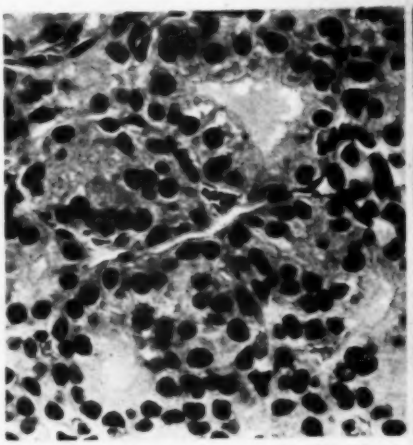

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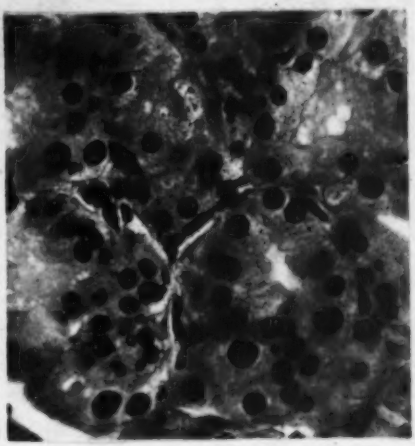

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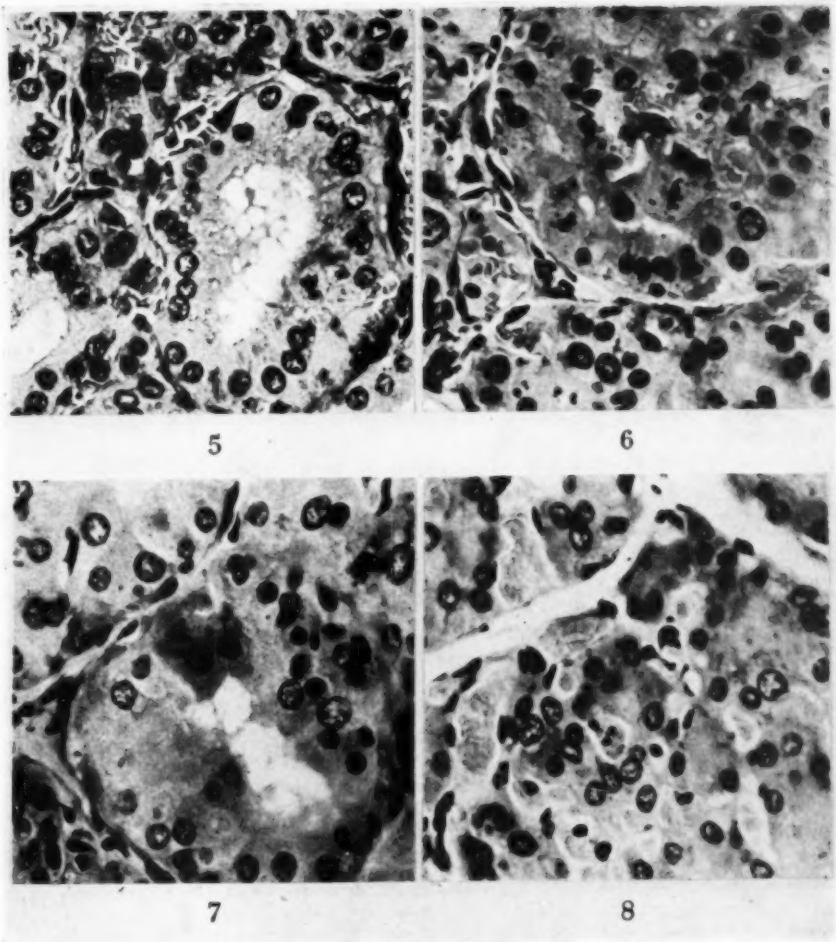

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TABLE III.—*Effect of Hemithyroidectomy on Thyroid Weight and Iodine Uptake after* I^{131}.

Number of rats.	Treatment.	Mean body weight (g.) + S. deviation of mean.	Mean weight (mg.) of residual thyroid lobe + S. deviation of mean.	*Thyroid uptake of* I^{131}. Mean thyroid activity in 1000 counts/min. + S. deviation of mean, 2½ hours after 10 μC ^{131}.
10	Hemithyroidectomized 9 days previously. No I^{131}	206±18	9·4±0·6	5·9±1·7
10	30 μC I^{131} 3 months previously. Hemithyroidectomized 9 days previously	213±15	7·3±1·0	5·2±1·2
4	30 μC I^{131} 3 months previously not hemithyroidectomized	191±30	15·4±1·2	5·5±2·6

intracytoplasmic colloid droplets. The follicle cells of the irradiated glands appeared larger and showed a greater variation in nuclear size than the unirradiated hemithyroidectomized controls (Fig. 3 and 4). Unusually large nuclei and occasional micronuclei were present in the irradiated glands. Additional changes seen only in the irradiated glands, especially centrally, were bizarre-shaped nuclei, examples of marked infolding of the nuclear membrane, multinucleated follicle cells containing two or more small nuclei, and degenerate nuclei whose chromatin was seen as pycnotic dots lying within the ghost of a nuclear membrane.

Experiment 4.

In our results so far, we had failed to detect any loss of ability of iodine uptake in the irradiated thyroids. We had found a decrease in thyroid weight and the development of nuclear anomalies under the stimulus of regeneration. We therefore designed the following experiment to see if irradiated glands would show a reduced ability to undergo hyperplasia induced by a standard short course of propylthiouracil. We were particularly interested to see the effect of increasing the time-interval after irradiation when applying the goitrogen.

We found in preliminary trials with varying strengths of propylthiouracil solution that " drinking water " containing 6 mg. propylthiouracil per 10 ml. was well tolerated and was effectively goitrogenic. We also found, by killing off pairs of rats at 2-day intervals, that their thyroids, on this régime, showed a rising mitotic index up to about 8 days, followed by a plateau lasting about 4 days and then a fall to practically nil by 24 days. We then set up a series of 5 groups of animals, totalling 90 male albinos 2½ months old. Each group contained 6 controls, 6 rats injected intraperitoneally with 10 μC I^{131} and 6 given 30 μC I^{131}. Except for the week following the injection of I^{131} the 18 rats in each group were housed in a single large cage. The goitrogenic stimulus was a 10-day course of 6 mg. propylthiouracil per 10 ml. in place of drinking water following an initial subcutaneous injection of a solution of 10 mg. propylthiouracil. The propylthiouracil was started 3 days after the I^{131} in Group (a), 21 days in Group (b),

48 days in Group (c), 81 days in Group (d) and 120 days in Group (e). After 10 days on the propylthiouracil the rats were killed by coal gas, their thyroids removed and fixed in Helly for 3½ hours, washed, weighed, embedded in wax and sectioned as in Experiment I. Mitoses were counted, using an oil-immersion objective, the whole width or length of each thyroid lobe being traversed. The number of mitoses per 5000 cells was counted in each gland making a total of 30,000 for the controls and 30,000 for the rats given 30 μC I^{131} in each group. Only cells in which the nuclear membrane had disappeared were registered as dividing, so that the mitotic count was virtually one of metaphases and anaphases, prophases being mostly classified as resting cells. In our counting we alternated the slides from controls and irradiated rats so as to keep our criteria as constant as possible.

The results summarized in Table IV show a remarkably consistent average weight of goitre in the control rats of Groups (a) to (e) varying from 55·5 mg. to 61·3 mg. in spite of the variation in the rats' average body weight: 245·8 g. to 376·7 g. and mitotic index: 60–208 mitoses per 30,000 cells. The average weight of the thyroids of all the irradiated rats was less than the controls. The most striking failure to respond to the goitrogenic stimulus was seen in the 30 μC rats 120 days after the administration of the I^{131}, Group (e). The diminution in goitrogenic response was evident in the 10 μC rats (42·5 mg. average thyroid weight), but did not alter significantly during the four months under study. A similar inhibition of goitrogenic response was produced by 30 μC but this became increasingly effective after 48 days when the average thyroid weight was 36·8 mg. It was 31·2 mg. following the goitrogen at 81 days and only 25·5 mg. at 120 days.

Histological examination of the thyroids showed the classical changes of hypertrophy and hyperplasia induced by goitrogens. Follicles were increased in number, colloid was grossly reduced, follicle cells were increased in height to above 15μ, many contained intracytoplasmic colloid droplets (Fig. 5). In addition to numerous mitoses, the nuclei in control glands showed a greater variation in size and shape than those of unstimulated thyroids and very occasional micronuclei. However, the nuclei of the irradiated glands (10 μC and 30 μC) showed an even more striking variation in size and shape. Micronuclei appeared more numerous. The anomalies seen in the regenerating hemithyroidectomized irradiated glands were present and all were more marked (Fig. 6, 7 and 8). At 120 days, when mitoses were reduced to a minimum, cell hypertrophy was still as much in evidence as in the control unirradiated glands. The follicles of the irradiated glands showed a gradual increase in cell size.

Normal and abnormal mitoses were present in all the irradiated glands. The latter consisted mainly of irregular arrangements of the chromosomes at metaphase and anaphase. Occasional cells were present containing clumps of scattered deeply staining chromatin, an appearance suggestive of death and the onset of degeneration of a dividing cell. Multinucleate follicle cells with small overlapping nuclei were common.

Groups (f) 7 controls and 7 irradiated and (g) 6 controls and 7 irradiated male albino rats have been added to Table IV from two separate (unpublished) experiments. Their treatment was similar to Group (e) except that the 14 animals in Group (f) were injected with colchicine 8 hours before they were killed. All confirm the markedly reduced goitrogenic response 4 months after 30 μC I^{131}. The mean thyroid weights were 28 and 32 mg. in the irradiated and 52 and 54 mg. in the unirradiated controls.

TABLE IV.—*Goitrogenic Response after I^{131}.*

Time after I^{131} injection at which propylthiouracil course was commenced.	Controls.			10 μC I^{131}.		30 μC I^{131}.			Ratio of mitoses in 30 μC rat thyroids to controls.
	Mean body weight in g. + S. deviation of mean.	Mean thyroid weight in mg. + S. deviation of mean.	Mitoses counted in 30,000 cells.	Mean body weight in g. + S. deviation of mean.	Mean thyroid weight in mg. + S. deviation of mean.	Mean body weight in g. + S. deviation of mean.	Mean thyroid weight in mg. + S. deviation of mean.	Mitoses counted in 30,000 cells.	
3 days (a)	245·8±15·6	59·3± 7·8	184	242·5±20·9	42·5±6·5	225·8±18·0	35·8±4·4	88	0·48
21 days (b)	279·2±33·9	55·5± 4·5	121	265·0±14·5	40·8±6·0	281·7±24·4	40·7±6·3	68	0·56
48 days (c)	343·0±17·2	59·0±11·5	208	331·0±13·5	47·2±9·4	330·8±17·2	36·8±4·4	58	0·28
81 days (d)	345·8±31·6	60·2±11·9	60	338·3±43·9	40·8±5·5	352·5±22·5	31·2±4·3	14	0·23
120 days (e)	376·7±36·7	61·3±10·3	172	413·0±36·2	41·0±8·2	390·0±35·6	25·5±4·7	6	0·035
132 days (f)	369·0±63·4	52·0±10·4	349 (colchicine)	—	—	380·0±62·7	29·0±8·8	30 (colchicine)	0·086
129 days (g)	364·0±16·4	54·0± 7·1	112	—	—	418·0±19·4	32·0±3·6	35	0·31

The mitotic index was less in all the irradiated rats than in the controls. It was reduced to a half of that of the controls at 3 days and 21 days in the 30 μC groups and to less than a third at 48 days and at 81 days. After 4 months, namely, Groups (e), (f) and (g), the results varied. The mitotic index was reduced in all. This was most striking in (e) and (f) in which it was reduced to less than one-tenth of the controls. In Group (g) it was only reduced to one-third.

Experiment 5.

The goitrogenic response 4 months after irradiation was tested in a similar way to Experiment 4. Half the animals were given I^{131} and half given external X-irradiation to the thyroid region by Dr. J. D. Abbatt. We are reporting the results in detail in a separate paper. However, we should like to note here that a similar degree of inhibition of goitrogenic response resulting from 30 μC I^{131} was obtained with a dose of 1000 roentgens of 190 kV X rays to the thyroid. (The diameter of the X ray beam was 13 mm. and the dose rate at the thyroid gland, taken as 8 mm. deep, was 150 r/min.)

DISCUSSION.

The impossibility of assessing accurately the radiation dosage to the thyroid from I^{131} because of variation between follicles in iodine uptake and turnover, radiation cross-fire at the centres of the lobules and escape at the periphery has been discussed previously (Feller, Chaikoff, Taurog and Jones, 1949; Maloof, Dobyns and Vickery, 1952; Doniach, 1953). The dosage range to the thyroids in rats which absorbed 20 per cent of 30 μC I^{131} was considered to lie between 3200 and 22,600 rads. (Doniach, 1953). The experimental findings reported above show that this irradiation produces a loss of thyroid weight which becomes more marked with time. The reduction in weight was definite after 3 months (Tables IA and IB). In previous experiments (Doniach 1950 and 1953) the thyroid weight was still further reduced 15 months after I^{131}. The loss of weight was not associated with any obvious loss of function. The animals grew normally, their smaller thyroids concentrated iodide normally (Experiment 2) and showed a 2-hour uptake of bound iodine quantitatively similar to the larger unirradiated glands. It would appear likely that the smaller irradiated thyroids maintain a euthyroid state by increased activity of their cells. This was borne out by the findings of an increased mean cell height and a diminution in colloid. On the other hand we expected to find that this hypertrophy would be associated with other evidence of a relative increase in circulating pituitary thyrotrophic hormone (T.S.H.). We hoped, therefore, to find an associated increase in T/S iodide ratio.

Our failure to do so suggests that this method was not sensitive enough to detect the increased thyroid activity manifested quite clearly in histological sections. The variation in T/S iodide ratio was large in the rats in any one group. Statistical evidence of an increase in iodide concentration might have been found if many more rats had been used.

The findings in Experiment 3 prove that even after hemithyroidectomy the residual lobe of the irradiated gland can still function adequately, i.e. bind iodine in a similar amount to a non-hemithyroidectomized irradiated animal or a hemithyroidectomized non-irradiated one. But histology shows that this is

done at the expense of a very marked cell hypertrophy. Moreover, disturbing nuclear anomalies and degenerative cells are seen which suggest that the irradiated thyroid might be reaching the limit of its compensatory faculties.

The results of Experiment 4 bring out a number of major points. The irradiated thyroid cells are capable of full hypertrophy; in this experiment definitely in response to an increased blood level of T.S.H. following a 10-day course of a potent goitrogen. On the other hand the ability of the irradiated thyroid to undergo hyperplasia is reduced since histology showed a lowered mitotic index. Furthermore, this reduction in goitrogenic response becomes more marked with time. The non-stimulated irradiated thyroid glands at 4 months averaged 18 mg. (Table IB). The goitrogen stimulated irradiated thyroids at 4 months averaged 25·5 mg. (Table IV), Group (e), the stimulated controls 61·3. Presumably the increase in weight from 18 to 25·5 mg. was due partly to cell hypertrophy and to hyperaemia, and possibly to an increase in cell number since there were 6 mitoses counted in 30,000 cells. Irradiation may inhibit hyperplasia directly by the prevention of mitosis or may so damage cells that they break down after entering mitosis. The histological findings in Experiment 4 of abnormal resting and dividing nuclei are consistent with a radiation effect. Some of the appearances noted favour the likelihood of cell degeneration following an abnormal mitosis. We do not know why this radiation response of inhibition of hyperplasia increases with time. Most radiation experiments on plant and mammalian tissues have dealt with the action of radiation upon proliferating cells. This contrasts with the present experiment in which the irradiation was administered to a resting tissue stimulated to proliferate many weeks later. Though our findings suggest that the longer the time-interval following irradiation the more hazardous is cell division, we have no reason to assume that breakdown in division accounts entirely for the thyroid's inability to grow.

It is difficult to explain why thyroid cells should be either increasingly less able to survive mitosis or else become increasingly unresponsive to a mitotic stimulus with the passage of time after exposure to irradiation. Billen, Stapleton and Hollaender (1953) found that following the X-irradiation of resting *Escherichia coli* the apparently non-viable bacterial cells showed a capacity, limited in time, for normal respiratory activity. The authors postulated that this might have been effected by a limited reserve of enzymes whose reformation had been inhibited by the X-irradiation. We might postulate along these lines that irradiation diminishes the ability of the thyroid cells to reform hypothetical enzymes which are gradually used up in time by normal cell activity and are essential for division.

Skanse (1948) studied the effects of nondestructive doses of I^{131} upon thyroid function. He used 5-day old cockerels primed with 3 daily injections of T.S.H. and tested the effects of 1, 10 and 50 μCI^{131} upon thyroid growth, collection of I^{131}, response to thiouracil and to T.S.H. The calculated radiation dose to the thyroid was about 1700, 13,000 and 60,000 rep respectively for the 1, 10 and 50 μC. Normal thyroid growth was significantly inhibited by the larger doses. Ability to take up iodine was not altered in the 10 μC chicks, but was decreased in the 50 μC group. A 10-day course of thiouracil started 26 days after the I^{131} produced an increase in thyroid weight in all groups, but the 50 μC group did not show any thyroid weight increase in response to a 10-day course of thiouracil started 38 days after the I^{131}.

Maloof, Dobyns and Vickery (1952) carried out concurrent functional and histological studies on rats injected with 1 to 300 μC I^{131} at intervals ranging from 2 to 18 months after the I^{131} administration. No impairment in body weight gain was noted in animals receiving 30,000 rep or less to the thyroid. There was a persistent increase in follicle cell height in all animals that had received a calculated maximum dose of 5800 rep or more to the thyroid. This was associated with a diminished thyroid gland weight but a normal ability to take up a tracer dose of I^{131}, 48 days after the initial irradiation. A 30-day course of thiouracil, instituted 64 days after the initial radiation showed a good goitrogenic response in the animals which had received 1800 rep to the thyroid, a diminished response in the 5800 and 30,000 rep-groups and no goitrogenic response at all in groups which had received 80,000 rep or more to the thyroid. The authors described bizarre nuclear changes in the follicle cells after irradiation, accentuated by thiouracil administration and noted the irradiated thyroids' retention of ability to undergo cellular hypertrophy. They thought that the failure of response to thiouracil suggested an impairment of cellular division.

Our results largely confirm these radiation effects following the appropriate dosage of I^{131}, in particular the reduction in thyroid weight, maintenance of normal body weight and thyroid ability to take up iodine, increase in follicle cell height and ability to hypertrophy, inhibition of ability to undergo hyperplasia and the formation of bizarre nuclear forms. We have extended Skanse's observation that time after irradiation enhances the lack of responsiveness to thiouracil and confirmed by mitotic counts the suggestion of Maloof *et al.* (1952) of an impairment of cell division.

Our findings show that the degree of goitrogenic response 4 or more months after irradiation of the thyroid is a sensitive and easily determined index of radiation damage. The test proved satisfactory for the quantitative comparison of the effects of external X rays with internal I^{131} irradiation.

The other test which proved simple but less sensitive was the measurement of the post-irradiation loss of weight of the non-goitrogen stimulated thyroids. The mechanism is presumably due to the same hyperplasia inhibition noted above, except that here we are dealing with a failure of the normal growth and renewal in time of thyroid tissue rather than the failure to respond to exogenously applied growth stimulus. This finding suggests that given enough time these irradiated thyroids might undergo a complete atrophy. It follows that some patients successfully treated with I^{131} for Graves' disease might nevertheless develop myxoedema due to post-irradiation thyroid atrophy many years after. The only function that we have found to be affected preferentially in rats by moderately damaging doses of I^{131} appears to be concerned with cell renewal; secretory function of the gland as a whole appears unchanged.

The capacity to divide is not lost altogether since the atrophying thyroid following 30 μC I^{131} grows occasional adenomata and when submitted to a prolonged goitrogenic stimulus may produce carcinomata (Doniach, 1953). The destructive dose of 100 μC I^{131} almost totally eliminated this capacity to produce adenomata (Doniach, 1953). However, Goldberg and Chaikoff (1952) obtained 7 thyroid carcinomas in 25 rats each given 400 μC I^{131} 18 months previously. Those post-irradiation cells which have retained the ability to undergo successful mitosis appear more liable than unirradiated cells to give rise to tumours when stimulated to division by a maintained rise in circulating thyrotrophic hormone.

SUMMARY.

Thyroid function tests were carried out on rats injected 3 months previously with 30 μC I^{131}, a dose considered comparable with that used in the treatment of Graves' disease. There was a loss of thyroid weight, an increase in follicle cell height, a normal T/S iodide ratio and a normal thyroid uptake of I^{131}. The residual lobe after hemithyroidectomy showed a similar I^{131} uptake, a smaller mass, and a greater cell hypertrophy than non-irradiated hemithyroidectomized controls. The irradiated glands showed bizarre nuclear forms. A series of rats was killed at the end of a 10-day course of propylthiouracil administered at varying time-intervals after 10 and 30 μC I^{131}. The goitrogenic response was less in the irradiated than in non-irradiated controls and diminished considerably with time in the 30 μC group. This impaired goitrogenic response was associated with a marked reduction in mitosis; cell hypertrophy was not reduced. The findings of abnormal mitoses, micronuclei and other bizarre nuclear forms favour the hypothesis that part of the inability to undergo hyperplasia is due to a radiation effect on the nucleus. Measurement of the diminished goitrogenic response 4 months after 30 μC I^{131} proved a sensitive test for radiation damage and was found applicable to the measurement of X-irradiation damage.

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A FURTHER INVESTIGATION OF THE RÔLE OF SKIN COMPONENTS IN CHEMICAL CARCINOGENESIS.

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PREVIOUS communications (Billingham, Orr and Woodhouse, 1951 ; Marchant and Orr, 1953) have presented results of experiments on transference of various skin layers and the effects of trauma during chemical carcinogenesis. From these experiments the following main points have emerged :

Pure epidermal or thin Thiersch grafts of carcinogen-treated skin transplanted to untreated body sites of the same mouse did not give rise to tumours. When such grafts were subsequently painted with croton oil, the number of papillomata which appeared was not significantly different from the number obtained by painting normal body skin with croton oil alone. Numerous tumours appeared on the original treated donor site. Thick Thiersch grafts and full-thickness grafts of carcinogen-treated skin yielded a small number of tumours after transplantation to sites in normal body skin. Large numbers of tumours appeared when a denuded carcinogen-treated area was left to resurface itself, or resurfaced with tail epithelium which had not received carcinogenic treatment. Reimplantation of grafts into the site from which they were cut in carcinogen-treated skin gave a number of papillomata which later regressed, but the number of persistent tumours was not increased above that of control animals.

The present experiments are further extensions of the above, and include some in which grafting procedures were carried out prior to carcinogen treatment.

CARCINOGENIC TREATMENT.

The standard treatment employed was as described in the previous reports, namely, once weekly applications of a drop of 0·3 per cent solution of 20-methylcholanthrene in acetone for 12 weeks. The animals used were adult white mice of mixed stock and both sexes.

OPERATIVE METHODS.

These have also been described in previous papers, grafting being done under aseptic conditions with nembutal anaesthesia. Grafts were bound in place by gauze impregnated with petroleum jelly wound firmly round the thorax of the mouse and covered with "Gypsona" plaster-impregnated bandage. The dressings were finally removed after 2 to 3 weeks.

Experiment P ; Transplantation of carcinogen-treated pure epidermis to a recipient area cut in croton oil-painted skin and vice-versa.

This experiment was done to find some indication whether or not tumours would arise on carcinogen-treated pure epidermis which had been transplanted

to a bed whose nature had been changed by previous application of croton oil. Orr (1938) showed that croton oil was not unlike the carcinogens in its histological effects on the skin, but very much slower than them in altering the connective tissue. The changes referred to are transformation of the collagen of the dermis into a fine-fibred, non-refractile type, alterations in the texture of the elastic tissue, and passive congestion of the sub-cutis.

Seven mice were painted once weekly for 24 weeks with 0·5 per cent croton oil in acetone on the left side of the thorax and with 0·3 per cent methylcholanthrene in acetone on the right side of the thorax, during the last 12 weeks of this period. Several thin Thiersch grafts were then taken from both treated sites on each animal, removing all the obviously thickened epidermis, and the attached dermis was removed after incubation with trypsin solution (Billingham and Medawar, 1951). The pieces of pure epidermis obtained were planted back on to the site contralateral to that from which they were derived.

A persistent tumour appeared on the carcinogen-treated epidermis grafted to the croton oil-treated site of one of the 7 mice after 143 days from operation. It was clinically malignant at 162 days from the operation and histologically was a squamous carcinoma. Two small papillomas appeared on another mouse, but they regressed before the mouse died.

On the carcinogen-treated site bearing grafts of croton oil-treated epidermis, tumours appeared in 6 of the 7 mice in from 18 to 162 days from operation (mean 92 $\pm$ 26 days). Five of the tumours were clinically malignant in 40 to 162 days (mean 99 $\pm$ 23 days) from operation.

The mice survived from 94 to 234 days from operation (mean 174 $\pm$ 19 days).

The single carcinoma which arose on the carcinogen-treated epidermis transplanted to a croton oil-treated site is interesting in that this particular tumour became clinically malignant within 3 weeks of its appearance, and by the same day a tumour had appeared on the carcinogen-treated site of the same animal which was malignant from its first appearance. This might indicate that the graft of carcinogen-treated epidermis from which the tumour arose already contained some irreversibly altered cells, and that some similar irreversibly altered cells remained behind in the roots of hair follicles to give rise to a malignant tumour at exactly the same time on the original treated site.

It would seem from this experiment that treatment of skin with croton oil does not modify the dermis to such an extent that carcinogen-treated pure epidermis transplanted to it will give rise to tumours in as great number or rapid time as if left on its original site.

Experiment Q: Transplantation of the dermis from carcinogen-treated skin to a bed cut in normal skin and natural resurfacing of the raw area on the carcinogen-treated site.

In this experiment 12 mice were used. The epidermal surface of the carcinogen-treated site was removed as thin Thiersch grafts and a pinch graft (full thickness) was then taken from the denuded area. The panniculus carnosus muscle and fat were then trimmed off and the graft rolled in animal charcoal for identification. Such grafts would be comprised of the lower part of the collagen layer of the dermis with a few hair follicle roots. The graft was then transferred to a site prepared on the opposite untreated side of the thorax by removing a

similar pinch of skin. Both sites were left to resurface themselves. The original carcinogen-treated site healed into a long scar.

A tumour appeared over the grafted dermis of one of the 12 animals after 38 days from operation and grew into a very large horn, but did not invade the deeper tissues. Histologically it consisted almost entirely of keratin. A papilloma appeared over another graft after 50 days, but it regressed after 120 days. The mice survived from 73 to 438 days after operation (mean 241 $\pm$ 30 days).

On the carcinogen-treated side of the animal 5 persistent tumours appeared on the scar and 4 outside it. The time of appearance of those on the scar was from 20 to 157 days after operation (mean 56 $\pm$ 25 days), and the time of appearance of those outside the scar was 68 to 200 days (mean 151 $\pm$ 31 days). The difference is significant $\left(\frac{D}{S.E.} = 2{\cdot}4\right)$.

Three of the tumours on the scar and 3 outside it became malignant, those on the scar after 37, 87 and 98 days (mean 74 $\pm$ 13 days). This difference is also significant $\left(\frac{D}{S.E.} = 8\right)$.

The appearance of a tumour on the grafted dermis of 1 animal in this group could be interpreted as indicating that the dermis of the carcinogen-treated skin has been altered in such a way as to be capable of inducing a tumour on regenerated epidermis. The epithelium responsible for the origin of this tumour could be derived either from the roots of hair follicles included in the graft, or from surface epithelium spreading in from the edge of the graft bed. It is impossible to choose with certainty between these two sources, but it is relevant to point out that histological examination of the remains of the grafted dermis revealed no relics of surviving follicles. A third possibility, of course, is that the dermal graft already contained at the time of operation an incipient tumour. If this were so, it is difficult to understand why it showed no evidence of infiltration.

The most interesting results of this experiment, however, are the time differences between appearance of tumours on the scar of the denuded area on the treated side of the animal and the appearance of tumours outside this scar. The significantly shorter time on the former supports the suggestion of Linell (1947) that deep trauma to a carcinogen-treated site speeds up the appearance and malignancy of tumours.

Some experiments were done to see whether grafting of skin prior to painting with a carcinogen would affect tumour production in any way.

Experiment R: Reimplantation of a pinch graft of normal skin and subsequent painting of the graft with carcinogen.

A pinch graft of body skin measuring about 1·5 cm. in diameter was cut from each of 18 mice. The grafts were then trimmed of fat and muscle and reimplanted, being marked round with animal charcoal. After 4 weeks, the completely healed grafts were painted with methylcholanthrene in acetone for 12 weeks. A control group of 15 ungrafted mice were painted at the same time with carcinogen.

Fourteen of the 18 mice which had skin reimplantations produced tumours, but regression occurred on 1 mouse. In another mouse the tumour was outside

the grafted skin and is not counted in the following results. On the 12 remaining mice, persistent tumours first appeared after 55 to 470 days (mean 237 ± 39 days) from the first painting with carcinogen. Of these 12 tumours, 8 appeared on the grafts themselves after an average of 220 days, and 4 appeared over the scar surrounding the grafts after an average of 272 days. All the tumours arising on the grafts themselves became malignant after an average of 262 days, and 3 of the 4 on the scars did so after a significantly longer average of 364 days. The mice survived from 141 days to 638 days (mean 351 ± 32 days).

Of the 15 control mice, 13 produced tumours 107 to 320 days (mean 175 ± 19 days) after the first painting with carcinogen. Twelve of these became malignant after 142 to 380 days (mean 256 ± 19 days). The mice survived from 196 to 438 days (mean 304 ± 22 days) from first painting.

The differences in tumour production between the grafted animals and the control animals are not significant except for a delay in malignancy of the 3 tumours appearing on the scars surrounding the grafts in the experimental animals. The number of animals is, however, too small to justify inferences.

In a further group of 8 mice, an attempt was made to cut thin Thiersch grafts of normal skin and to reimplant only the pure epidermis before painting with the carcinogen. This operation is too difficult technically due to the thinness of normal mouse skin, but at least one could say that in these animals there was trauma at the dermal level prior to carcinogenic treatment.

Of these 8 mice, all produced persistent tumours on the traumatised site in from 105 to 320 days (mean 206 ± 29 days). All became malignant after 178 to 363 days (mean 280 ± 25 days) from first painting with carcinogen. The mice survived from 217 to 448 days (mean 342 ± 34 days) from first painting. The differences between these mice and the controls are not significant.

Experiment S : Transplantation of full thickness ear skin to a bed cut in normal body skin and subsequent painting of the grafts with carcinogen.

Fifteen mice were used. The skin of both sides of the ears was cut through 3 or 4 times from the base to the periphery and stripped with fine forceps, leaving only the central cartilage. The detached ear skin was then transplanted, in about 6 pieces, to a thoracic bed prepared by removing thick strips of body skin and dusting with animal charcoal. The grafts healed quickly, retaining the character of ear skin. The area covered by the healed grafts was 3 to 4 sq. cm. Six to 7 weeks after grafting, weekly painting with methylcholanthrene in acetone were given for 12 weeks. A group of 11 ungrafted mice was painted on the thorax at the same time to serve as controls.

Persistent tumours appeared on 14 of the 15 grafted mice. On 1 animal the tumour appeared outside the grafted area 363 days after the first painting with carcinogen. In the other 13 mice tumours arose on the grafted area after 96 to 260 days (mean 154 ± 14 days) from the first painting. Most of the mice developed several tumours some of which regressed, but at least one of them became malignant on each mouse after 119 to 320 days (mean 206 ± 17 days). Very few of the tumours arose on the actual grafts of ear skin, and only 1 of these became malignant. Most of the malignant tumours developed over the scars between two grafts, but about one-third of them arose at a slightly later average time over the zone of healing between the ear skin grafts and the normal body

skin. The mice survived from 118 to 460 days (mean 260 ± 21 days) from the first painting.

There was no significant difference in tumour production between the grafted mice and the controls. In the latter persistent tumours appeared on 9 of the 11 mice after 76 to 270 days (mean 128 ± 20 days) from first painting. All of them became malignant in 91 to 314 days (mean 198 ± 22 days). The mice survived from 153 to 460 days (mean 282 ± 35 days) after the first painting.

The results of this experiment would seem to show that ear skin itself is refractory to the production of tumours by methylcholanthrene in acetone when transplanted to a site in body skin. (We had previously found this to be so with ear skin *in situ*). Most of the tumours arose between the pieces of ear skin.

Experiment T: Reimplantation of thin Thiersch grafts of normal skin and subsequent painting with croton oil.

This experiment was done as a control for Experiment J (Marchant and Orr, 1953) in which pure epidermis was transplanted from a carcinogen-treated site to a site in untreated skin and then painted weekly with croton oil for the remainder of life. In addition to a small number of papillomata, such as are obtained by croton oil painting alone, a single carcinoma occurred on such epidermis in one of 37 mice. It was considered desirable to know whether modification of the dermis of normal skin as a result of the grafting operation would affect the tumours yielded by subsequent croton oil painting.

Since it is extremely difficult to obtain thin Thiersch grafts or pure epidermal grafts from normal body skin in the mouse, the skin was treated once or twice before grafting with croton oil to induce hyperplasia. Thin Thiersch grafts were then cut from such skin in 24 mice and reimplanted, being marked with animal charcoal. After about 4 weeks, weekly paintings with 0·5 per cent croton oil in acetone were commenced. A control group of 9 animals received similar paintings, but no grafting operation.

Of the 20 grafted animals, 4 developed papillomas after 272 to 418 days (mean 344 days). Only 1 of these was actually on a graft, the other 3 being on surrounding skin. One of the latter regressed in 40 days. The survival of these mice was from 177 to 559 days (mean 403 ± 23 days).

Of the 9 control mice, only 1 developed a papilloma after 340 days. The survival of these mice was from 138 to 454 days (mean 239 ± 42 days).

We include here the final results for the control animals in the previous Experiment J (Marchant and Orr, 1953). In this experiment 36 mice received once weekly paintings of croton oil without any previous treatment. Persistent tumours appeared on 7 of them in 247 to 540 days (mean 337 ± 41 days) from first painting. None became malignant. The mice survived from 212 to 676 days (mean 388 ± 23 days).

It will thus be seen that there was no significant difference between the two groups of Experiment T and the control group of Experiment J with regard to tumour yield or time of appearance of tumours. It may be concluded that preliminary Thiersch grafting does not modify the dermis in such a way as to affect the yield of tumours by croton oil painting.

The general trend of the results of these and the previously reported experiments has been to suggest that the tumours which arise in carcinogen-treated

skin are not determined by changes in the superficial epidermis itself, and that the major precancerous factors are located in the deeper layers. Reasons have been given for attaching importance to the connective tissue and other stromal elements, but there still remains the possibilty that the effective source of the tumours and site of action of the carcinogen may be the epithelium of the hair follicles. It occurred to us that by using the technique of co-carcinogenesis (Berenblum and Shubik, 1947) and new born mice without penetrated hairs or sebaceous glands, an answer might be obtained to this question.

Experiment U : Single painting with carcinogen on day of birth, or on seventh day after birth followed by croton oil to develop the tumours.

Two groups of mice were used in this experiment. The first, consisting of 9 animals, were given one painting of methylcholanthrene in acetone on the side of the thorax on the day of birth (before hair follicles had developed—Gibbs, 1941) and immediately returned to their mothers. The second group of 15 animals received a similar painting on the 7th day after birth (when hair follicles had developed) and were returned to their mothers. When the mice were 4 weeks old, weekly paintings of 0·5 per cent croton oil in acetone on the same site were commenced.

Of the 9 mice painted on the day of birth, 4 developed persistent tumours. One of these was a mammary tumour which arose 520 days after the methylcholanthrene painting. The other 3 were papillomas arising after 275, 368 and 464 days (mean 369 days). None of these papillomas became malignant. The mice survived from 193 to 568 days (mean 489 $\pm$ 44 days).

Of the 15 mice painted with carcinogen on the 7th day after birth, 10 developed tumours after 89 to 460 days (mean 192 $\pm$ 35 days). Six of the tumours became malignant after 280 to 490 days (mean 348 $\pm$ 32 days). The appearance of tumours on these mice was significantly quicker than in the mice painted with carcinogen on the day of birth. Their lives were significantly shorter, the survival being from 130 to 518 days (mean 358 $\pm$ 33 days).

It will be seen that tumours developed much more infrequently and slowly on the skin which received its application of carcinogen before the development of hair follicles than on the skin to which carcinogen was applied after hair follicles had developed. In fact, the time taken by the former is no different from the time taken by croton oil alone to elicit tumours in the control mice for Experiment J. In other words, carcinogen applied to mouse body skin on the day of birth is ineffective in producing carcinomas. This could be interpreted to mean that the presence of hair follicles in the skin is required, possibly as a mode of entry and storage of the carcinogen, in order that tumours should be elicited. But it is possible that the mothers of the new-born mice lick off all the applied carcinogen before it has time to act. In order to test this point, some new-born and some 7-day old mice were painted with methylcholanthrene, returned to their mothers, and observed at intervals under an ultra-violet lamp to see how long the skin remained fluorescent in each case. The following results were obtained : Fluorescence was considerably faded from the newborn mice after 4 hours and completely disappeared after 24 hours. One baby which was kept in the warm away from the mother was still alive and showed bright fluorescence after 24 hours. The 7-day old mice were still brightly fluorescent after 24 hours, and faintly so after 3 days.

DISCUSSION.

The results of Experiment U in which no malignant tumours were obtained after a single application of carcinogen to the skin of new-born mice, followed by painting with croton oil, confirm the previous experiments of Suntzeff, Carruthers and Cowdry (1947). They obtained no tumours after application of 0·6 per cent methylcholanthrene in benzene to new-born mice and considered failure might be due to the thickness of the epidermis (5 to 7 cells) preventing penetration of the carcinogen, or to the scarcity of penetrated hair follicles and the lack of sebaceous glands. We have also considered the fact that the carcinogen might be licked off by the mothers very rapidly, and have found it does disappear during the first 24 hours. The thickness can probably be discounted, since tumours will develop after carcinogen application to epidermis rendered hyperplastic by such agents as croton oil.

The necessity of hair follicles in the mechanism of skin carcinogenesis is suggested by the experiments of Lacassagne and Latarjet (1946). They gave repeated applications of methylcholanthrene in acetone to ultra-violet burns on the skin of new-born and adult mice. When the applications were begun 10 days after the burning (at a time when there had been some regeneration of hairs and sebaceous glands) tumours appeared on the scar. If the applications were begun on the day following the burn, healing was unimpaired, but no hair follicles appeared on the scar and nor did any tumours. From our almost invariable failure to obtain tumours on grafts of pure carcinogen-treated epidermis transplanted to untreated sites, we can say with some confidence that, at a stage when the carcinogen-treated site is capable of epithelial neoplasia, this potentiality is not an intrinsic property of the cells of the superficial epidermis. Therefore, if the carcinogenesis is determined by a primary intrinsic change in epithelial cells alone, the parent cells of the tumour might be those of the hair follicles or sebaceous glands.

There is no doubt that the hair follicles and sebaceous glands represent an important mode of entry of the carcinogen into the skin. Simpson and Cramer (1945) for example, were able to show by fluorescence studies, that methylcholanthrene reaches the sebaceous glands almost immediately after superficial application. At a later stage some of it is found in the dermal connective tissues, but fluorescence persists in the sebaceous glands for a considerable time. The cells of the hair follicle are thus exposed to the direct action of carcinogen for a greater time and in greater concentration than those of the superficial epidermis, and it is impossible to exclude them as the actual site of carcinogenesis.

There are, however, some difficulties in accepting the hair follicles as the source of most of the tumours. In the early stages of chemical carcinogenesis with methylcholanthrene, complete epilation of the treated area of skin occurs after one or two weeks, and histological examination at this stage shows disappearance of most or even all of the hair follicles. Regeneration subsequently takes place, and with continued treatment there follow cycles of epilation and regeneration (Orr, 1938). Thus the hair follicles which originally receive the carcinogen do not survive to the time when tumours may be expected to develop. In the case of experiments where a series of applications of carcinogen is made, there are of course opportunities for the later generations of hair follicles to come

into direct contact with carcinogen, but this is not possible in cases where carcinoma is induced by a single application of carcinogen followed after an interval by serial applications of co-carcinogen. Moreover, only a small proportion of the tumours induced by acetone solutions of methylcholanthrene show any histological evidence of trichoepitheliomatous structure. The single horny papilloma which arose over transplanted carcinogen-treated dermis (Experiment Q) was confined to the superficial epidermis and showed no connection with any structures in the dermal graft region. It is just possible, of course, that some part of the regenerated epidermis was derived from hair follicles in the dermal graft.

It is certain that progressive changes do occur during skin carcinogenesis in tissues other than the treated epidermis (Doderlein, 1926; Orr, 1938; Howes, 1946). These changes include conversion of the parallel bundles of collagen fibres to loosely-packed disorientated fibrils, alterations in the elastic tissue and passive congestion of the blood vessels. Whether any of these changes are significant in the induction of tumours on the carcinogen-treated site is not established, but the possibility must continue to be taken into consideration.

The fact that tumours were very rarely found on grafts of ear skin transplanted to a site in body skin is rather mysterious. Tumours arose between the grafts. The graft-beds were prepared by removing fairly thick strips of skin but probably leaving behind some patches of the deeper collagen layer with the bases of some hair follicles from which epidermal regeneration could take place. It should, however, be noted that the anatomical features by which ear skin can be distinguished depend on the presence of the dermis. It is therefore impossible to know whether epidermis between the grafts was derived from the ear-skin grafts themselves, or from the body skin.

The accelerated appearance of tumours on the denuded carcinogen-treated site in Experiment Q, and the delay in malignancy of tumours on the scars surrounding pinch grafts in Experiment R, are of interest in that they lend support to the findings of Linell (1947) that whereas deep trauma to skin previously treated with carcinogen provoked an increase in tumour yield, no similar phenomenon was noted if the trauma preceded the chemical treatment.

SUMMARY.

Stock outbred white mice were used, and the carcinogenic treatment employed in most experiments was painting with 0·3 per cent 20-methylcholanthrene in acetone once weekly for 12 weeks. After such treatment, a high incidence of local skin tumours may be expected.

Pure superficial epidermis was removed from the carcinogen-treated site and exchanged with superficial epidermis from the contralateral side, which had received 24 previous weekly paintings with 0·5 per cent croton oil in acetone. Transplants of carcinogen-treated epidermis to croton oil-treated sites yielded a tumour (carcinoma) in one out of 7 mice; transplants of croton oil-treated epidermis to a carcinogen-treated site yielded tumours in 6 out of 7 animals (5 carcinomata, 1 papilloma).

Transplantation of carcinogen-treated dermis alone to a site in normal skin yielded 1 papilloma in 12 animals. On the donor site, tumours appeared and became malignant more quickly on the scar than outside it.

Reimplantation of pinch grafts before carcinogen treatment led to a signific-

antly slower appearance of malignancy in tumours developing on the scar surrounding the graft than on the grafts themselves.

Ear skin transplanted to the thorax was refractory to subsequent carcinogen treatment. Tumours appeared between the individual grafts and also at the edge of the grafted area.

Carcinogen applied once to the skin of new-born mice followed by croton oil paintings does not produce more tumours than croton oil alone, but when the same treatment was applied to 7-day-old mice, tumours appeared on many animals and several became malignant. Fluorescence disappears from the painted new-born mice in a few hours when they are returned to their mothers, but it is visible on the 7-day-old painted mice for at least 3 days.

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THE CARCINOGENIC ACTION OF CITY SMOKE.

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IN his presidential address to the Chemistry Section of the British Asssociation at the Liverpool meeting in 1953, Clemo described the chemical separation of three fractions from the smoke emitted from chimneys in the vicinity of this laboratory, two of the chimneys concerned belonging to this Infirmary (Clemo, 1953).

These fractions, together with two samples of extracts obtained from Diesel engine fumes, have since been tested on mice. Many of the mice which were available at the time the tests were suggested were already rather old for an experiment which could last at least 12 months, and they belonged to several inbred strains, some of which were less suitable than others for testing for skin carcinogenicity. But, rather than lose time in breeding more suitable mice, the experiment was begun with these animals in October, 1953, in the hope that some indication of the properties of the various crude fractions might be obtained even with this somewhat unsatisfactory material, and later a more complete test could be carried out on the most suitable strain of mice with the pure substances in the fractions. This hope was justified; two of the fractions were found to possess definite carcinogenic properties.

MATERIAL AND METHODS.

Smoke fractions A, B and C, obtained as described by Clemo (1953), were each diluted to a 1·0 per cent solution in benzene and applied to the interscapular region three times weekly, with two strokes of a No. 4 brush. Painting was continued until death or until definite malignant tumours appeared. The mice were not shaved before treatment. Two fractions obtained from Diesel engine fumes were similarly applied, and recently experiments with a third Diesel fraction have been begun.

The treated mice (male and female), 83 in all, belonged to the following inbred strains: CBA/Mr, A/Gr_f, C57BL/How, C57BL/Gr, GFF, GFF_f, A2G and $C3H_f$. In addition, 21 mice from these strains were used as controls and painted with benzene only.

Sections have been made of all skin papillomata and epitheliomata and examined microscopically; one tumour was found to be a mixed sarcoma-epithelioma.

The results are given in Table I, which also shows the ages of the mice at the commencement of treatment, and the duration of treatment. The data for mice of the same strain which gave negative results have been grouped together.

TABLE I.—*Experimental Data and Results.*

Smoke fraction.	Treated mice. Number.	Strain.	Sex.	Age at beginning of treatment (months).	Duration of treatment (months).	Results of treatment. Number of lung nodules.	Skin papilloma.	Skin epithelioma.	Earliest visible skin reaction (months).
A	6	CBA	F	3·0–4·5	7·0–9·5	0	–	–	–
	4	CBA	M	4·0	4·5–9·0	0	–	–	–
	4	A/Grf	F	6·0	6·5	1	–	–	–
					6·5	2	–	–	–
					8·0	0	–	–	–
					8·5	2	–	–	–
B	4	CBA	F	7·0	7·0–8·0	0	–	–	–
	1	CBA	F	7·0	11·5	3	–	–	–
	4	C57BL/Gr	M	2·5	12·0	0	+	+	9·0
					13·5*	0	+	+	9·0
					13·0	0	+	+	11·0
					13·5	1	+	–	11·0
	6	A/Grf	F	4·5	8·0	0	–	–	–
					10·5†	4	+	–	10·5
					11·5	3	+	+	8·0
					12·0	3	+	+	8·0
					12·5	0	+	–	11·0
					13·5	4	+	–	13·5
C	5	CBA	M	9·0–9·5	7·0	0	–	–	–
	2	A/Grf	F	7·0	2·0	1	–	–	–
					3·0	0	–	–	–
	4	A/Grf	F	6·0	6·5–7·5	0	–	–	–
	5	C57BL/How	F	4·0	7·5	0	–	+	6·0
					12·0	0	+	+	9·0
					12·0	0	+	+	9·0
					13·5	0	+	+	8·5
					13·5	0	+	+	10·0
					13·5	0	+	+	10·0
Diesel (first fraction)	3	A/Grf	F	2·0	7·0–8·0	0	–	–	–
	1	A/Grf	F	2·0	9·0	1	–	–	–
	2	GFF	F	2·0	6·5–8·5	0	–	–	–
	3	GFFf	M	1·5	6·0–8·0	0	–	–	–
	3	C3Hf	M	1·5	7·0–9·0	0	–	–	–
	4	GFF	M	2·0	6·0–9·0	0	–	–	–
	1	C57BL/Gr	M	2·0	9·5	0	–	–	–
	1	A2G	M	1·5	8·0	4	–	–	–
	3	CBA	F	3·0	10·0	0	–	–	–
Diesel (second fraction)	1	CBA	F	2·5	12·0	0	–	–	–
	1	CBA	F	2·5	14·0	0	–	–	–
	3	CBA	F	2·5	15·0‡	–	–	–	–
	1	C57BL/Gr	F	4·5	3·0	0	–	–	–
	1	C57BL/Gr	F	4·5	9·0	0	–	–	–
	4	C57BL/Gr	F	4·5	15·0‡	–	–	–	–
	6	GFFf	F	4·5	3·0–11·0	0	–	–	–
Controls	4	CBA	M	2·5	6·5	0	–	–	–
	1	CBA	M	2·5	10·5	1	–	–	–
	4	C57BL/Gr	M	1·0	14·0‡	–	–	–	–
	1	GFF	M	1·0	9·0	0	–	–	–
	5	A/Grf	M	2·0	6·0	0	–	–	–
	6	GFFf	F	1·0	5·5–8·5	0	–	–	–

Indices * = metastases in axillary lymph node.
† = forestomach papilloma present.
‡ = mice still living.

DISCUSSION.

The carcinogenic nature of soot was first recognised in 1775 by Pott, who described chimney-sweeps' cancer of the scrotum (Pott, 1775), but the experimental production of cancer by this substance was delayed until 1922 when Passey obtained malignant skin tumours in mice with an ether extract (Passey, 1922). So far as can be ascertained, no substance appears yet to have been isolated in a pure form from domestic soot. Goulden and Tipler (1949), by means of fluorescence spectroscopy, have identified 3 : 4-benzpyrene as one of its components and, using the same method, Waller (1952) has detected this same carcinogen in samples of smoke drawn from the air at eight different towns, and also in the deposit in the exhaust pipes of two diesel compressors.

In the present investigation three chemical fractions were prepared from chimney smoke and tested as described. These tests show that substances with a carcinogenic action on mouse skin are present in Fractions B and C, and it is suggested that there must be at least two different chemicals. C57BL mice were used in both tests, and from a comparison of their reactions Fraction C appeared to be more potent. Not only did it produce the earliest skin tumour (a very rapidly growing epithelioma) at 6 months, but the growths were more extensive and became malignant in all 6 mice. With Fraction B, one mouse which died after 13·5 months' painting did not produce a malignant growth. Although A/Gr$_f$ mice were also used to test both fractions, those treated with Fraction C were too old when treatment began and survived for only 6·5 to 7·5 months, scarcely a long enough period to produce even a wart ; with Fraction B, the earliest wart in this strain was seen after 8 months' painting, but one mouse of the strain died after 8 months' painting with no visible skin reaction.

An interesting feature of this group of A/Gr$_f$ mice treated with Fraction B was that 4 of the 5 animals had multiple lung nodules, and 1 had quite a large forestomach papilloma. Strain A is noted for its high incidence of spontaneous lung adenomata and is also very susceptible to induced lung tumours ; the number of lung nodules produced in individual mice has long been used as a criterion of the potency of a carcinogen (Andervont, 1937*a* ; Shimkin, 1940). In the A/Gr$_f$ mice treated with Fraction C only one solitary lung nodule was seen, which could have been spontaneous in a mouse 9 months old. Spontaneous forestomach papillomata are rare in Strain A mice in this laboratory ; in 3 years in several hundred mice there have been 3 instances, an A2G mouse 18 months old, and 2 A/Gr$_f$ mice of 14 and 15 months ; the present example in a 15-months old A/Gr$_f$ mouse was larger than these spontaneous cases and proved when sectioned to be malignant. An increased incidence of forestomach papillomata has been shown to be connected, in other strains of mice, with the carcinogenic action of a chemical (Miller and Pybus, 1955).

One CBA mouse painted with Fraction B had 3 lung nodules, an unusually large number for a mouse of this strain 18 months old. The strain has a lung tumour incidence of about 6 per cent, tumours usually being solitary, but multiple nodules occur under the influence of a carcinogen (Miller and Pybus, 1954). A small lung carcinoma was found in a C57BL/Gr mouse (also painted with Fraction B) ; this strain is highly resistant to lung tumours and no spontaneous lung tumour has been seen in the 3 years during which the strain has been bred in this laboratory.

Multiple lung nodules were found also in an A2G mouse painted for 8 months with the first Diesel fraction; the presence of 4 nodules in this 9·5-months old animal suggests the presence of a carcinogen in this fraction, but all the other mice in this group gave negative results. Although only young mice were used in this test, none survived more than 10 months' treatment (the CBA mice) and the majority, 11 out of 21, only 8 months or less. The results are therefore not entirely conclusive.

Neither were the results obtained with Fraction A conclusive; none of the mice survived more than 9 months' treatment, and skin reactions were negative. The A/Gr_f mice were 6 months old when first painted; lung nodules appeared in 3 of the 4, and in 2 of these there were 2 nodules; these mice were 12·5 and 14·5 months old at death and there is a strong possibility that the lung tumours were spontaneous.

From the present experiment, C57BL mice seemed to be more susceptible than A strain mice to the induction of skin tumours, the skin reactions in the C57BL mice painted with Fraction B being much more extensive than in the A/Gr_f. It has long been known (Andervont, 1937*b*) that they were much less susceptible than Strain A to the induction of lung tumours. It has recently been shown (Poel and Kammer, 1954) that C57BL mice were the most susceptible of 3 strains to the percutaneous application of a carcinogen.

In addition to the fact of strain specificity there is also the question of tissue specificity. It has been shown by Andervont and Shimkin (1940) and by Rask-Nielsen (1950) that different tissues react differently to the same carcinogen and that certain compounds are specific for certain tissues. The present experiments were concerned not with pure chemical substances but with a mixture of as yet unidentified chemicals. It may well be that more than one carcinogen is present, and that while one may be specific for skin, another may act more readily on the lungs, and that one strain of mice may be more susceptible to one carcinogen than to another.

Of the strains of mice used in this work, the GFF, GFF_f and CBA seemed intolerant of benzene alone, as well as of the smoke fractions in benzene solution. For percutaneous administration, Strain A males, or females freed of the milk factor by fostering, would be a second choice to the C57BL strain, but owing to their susceptibility to kidney disease they should be used as young as possible and are not suitable for the testing of the less potent carcinogens where painting may continue for many months. A further reason for using very young Strain A mice is of course their susceptibility to spontaneous lung tumours; the induced tumours should appear before the age of development of spontaneous tumours in order to obtain a clear-cut result, but the presence of multiple lung nodules is a strong indication of possible carcinogenic action.

SUMMARY.

Three chemical fractions, A, B and C, separated from industrial smoke, and two from Diesel engine fumes have been tested, by percutaneous application, for carcinogenic action on mice.

Fractions B and C produced malignant tumours as well as papillomata of the skin. Fraction C was possibly the more potent.

Multiple lung nodules appeared in five mice painted with Fraction B and in one mouse treated with a Diesel fraction. A forestomach epithelioma was found in one mouse painted with Fraction B.

Skin tumours were induced most readily in the C57BL strain, and lung tumours in two sub-lines of Strain A.

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THE INCIDENCE OF SPONTANEOUS AND INDUCED FORESTOMACH TUMOURS IN MICE OF TWO INBRED STRAINS AND THEIR RECIPROCAL HYBRIDS.

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IN this communication data are given of the occurrence of spontaneous tumours of the forestomach in the inbred strains of mice, CBA and NBT, and in the F_1 and succeeding generations of their reciprocal hybrids, and of the effect of the subcutaneous administration of methylcholanthrene upon the incidence of the same types of neoplasm in these same inbred and hybrid strains.

MATERIAL.

As previously described in detail (Miller and Pybus, 1954*a*), the inbred NBT and CBA strains were crossed reciprocally to give the NBT/CBA (or NC) and CBA/NBT (or CN) hybrid strains. Half of the F_1 mice each received one subcutaneous injection of 1·0 mg. methylcholanthrene in 0·1 c.c. sesame oil at the age of 2 months. These injected mice and their descendants, similarly injected, were bred from, brother to sister, for a total of 10 generations, and a further 2 generations of uninjected mice were bred, to give a total of 12 generations of MNC and MCN hybrids. The other half of the F_1 mice were not injected but were bred from, brother to sister, for a total of 12 generations, all uninjected, of NC and CN hybrids; these served as controls. In the MNC and MCN groups from F_5 onwards there were some mice, offspring of injected animals which early produced tumours at the site of injection, which were themselves neither injected nor bred from; these together with the whole of F_{11} and F_{12} formed the uninjected MNC and MCN groups and served as further controls.

Mice of the NBT and CBA strains were injected to give the M/NBT and M/CBA groups, and one uninjected generation (M/NBT F_1 and M/CBA F_1) was raised from them. Controls for the M/NBT and M/CBA groups were provided by mice of these inbred strains which were born during the period of the experiment.

In previous communications the incidences of tumours at the site of injection (Miller and Pybus, 1954*a*), of lung tumours (Miller and Pybus, 1954*b*) and of mammary tumours (Miller and Pybus, 1954*c*) in this same material have been described.

The experiment was begun in October, 1945, but no stomachs were opened until December, 1946, when a large squamous epithelioma was found in the stomach of an injected F_1 animal. The stomach of every mouse dying after that date was opened and examined carefully for benign and malignant tumours. All post-mortems made before that date have been excluded from the present report.

Throughout the following pages the word " tumour " without qualification refers to forestomach tumours only. Tumours were also found in the glandular part of the stomach, and will be described at a later date.

The forestomach tumours were of two main types, papillomata and gross malignant tumours. The papillomata varied in size from the smallest visible sessile or pedunculated nodule to quite large sessile or pendunculated arborescent masses half-filling the forestomach, and in number from solitary tumours to 7 or more in one stomach. Sections were cut of many of those found early in the experiment and, while the majority proved to be benign, a number showed early malignancy in the downgrowth of the epithelial cells through the muscularis mucosae. As it became impossible to examine microscopically every papilloma no attempt has been made to classify them as malignant or benign.

In addition to these papillomata there occurred a few grossly malignant tumours, the largest of which formed large solid masses in the abdomen. These were sectioned ; and while some were pure squamous epitheliomata, others were pure spindle-celled tumours and still others were found to consist of a mixture of both. Lateral invasion of the glandular area was seen, so that sometimes the main tumour mass appeared to be in the pyloric end. Extensive infiltration and metastases were sometimes found throughout the peritoneum and chest ; in one case there was invasion of a lymph node on the oesophagus.

Adequate descriptions and illustrations of benign and malignant forestomach tumours in mice, with which the present instances are in complete agreement, have been given by Stewart (1941), Collins, Gardner and Strong (1943), Stewart and Lorenz (1949), Bagshaw and Strong (1950), and Peacock, Beck and Chalmers (1953). No sarcomata of the forestomach were seen in their feeding experiments by Stewart and Lorenz (1949), but were obtained after remote injection by Bagshaw and Strong (1950), who doubted the sarcomatous nature of the tumours, and by direct injection by Firminger and Stewart (1951).

Since forestomach tumours, in common with tumours of the lungs and of other internal organs, were found only at autopsy, and since papillomata appeared to be of very slow growth, the age at which they developed cannot be stated, and " tumour age " became synonymous with the age at death. Thus " tumour age " will seem to be earlier in shorter-lived strains like the NBT than in those of longer life like the CBA, and earlier in males than in females if the former died at an earlier age than the latter. " Age at death " probably approximates most closely to " tumour age " in the injected mice, where more mice were dying in the earlier age groups, but the spontaneous type is essentially a tumour of middle and old age.

The following tumour incidences are based on the effective numbers of mice forming each group, i.e. the number living to the age of discovery of the earliest tumour in that group.

RESULTS.

A. *Pure Strains.*

1. *Spontaneous tumours.*

As in other respects, so the NBT and CBA strains differed in the incidence of spontaneous forestomach tumours, all of which were papillomata. Table I gives the tumour incidences and ages. In the NBT strain 28 females in 240 and 7

TABLE I.—*The Incidence of Forestomach Tumours in Control NBT and CBA Mice, in Mice of the Same Strains Receiving One Subcutaneous Injection of 1·0 mg. Methylcholanthrene, and in the One Uninjected Generation Inbred from these Injected Mice.*

Strain.	Sex.	Earliest tumour (months).	Effective number of mice.	Tumour mice. No.	Tumour mice. Percentage.	Tumour mice. Age at death (months). Range.	Tumour mice. Age at death (months). Average.	Non-tumour mice. Age at death (months). Range.	Non-tumour mice. Age at death (months). Average.
NBT	F	9·0	240	28	11·7	12·0–23·0	16·4	9·0–24·0	14·2
	M		214	7	3·3	9·0–17·0	14·0	9·0–22·0	12·3
					7·7		15·8		13·3
CBA	F	22·0	172	4	2·3	22·0–28·0	25·3	22·0–44·0	26·7
	M		109	0	0·0	—	—	22·0–34·0	25·3
					1·4				26·1
M/NBT	F	10·5	22	17	77·3	10·5–17·5	14·7	11·0–16·0	13·8
	M		6	0	0·0	—	—	10·5–12·5	11·4
					60·7				12·5
M/CBA	F	18·0	4	4	100·0	20·0–27·0	23·0	—	—
	M		7	1	14·3	18·0	18·0	20·0–26·0	22·3
					45·5		22·0		
M/NBT F_1	F	11·0	68	20	29·4	11·0–22·5	17·7	11·0–21·0	16·5
	M		66	10	15·2	11·5–19·0	14·2	11·0–18·5	13·9
					22·4		16·4		15·1
M/CBA F_1	F	20·0	162	7	4·3	20·0–30·0	27·0	20·0–34·0	26·1
	M		109	3	2·8	20·0–24·0	21·7	20·0–25·0	22·8
					3·7		25·1		24·7

males in 214 had these tumours; as the males died 2 months earlier than the females, the sex-difference in tumour incidence, which was significant,[1*] could be due to the shorter life of the males. Non-tumorous mice of both sexes died on an average 2 months earlier than tumorous mice; the percentage tumour incidences of 11·7 (females) and 3·3 (males) may not therefore be a complete expression of the strain susceptibility.

In the CBA strain only 4 tumorous mice, all females, were seen. Non-tumorous males and females lived as long as, or longer than, the tumorous. The CBA strain therefore had a much lower degree of susceptibility than the NBT, the difference being significant,[2] and tumours were found much later than in the NBT.

2. *Induced tumours.*

Tumours were found in the M/NBT group in 17 of the 22 females living to 10 months and over, autopsied after December, 1946. Only 6 males fulfilled these requirements and none had tumours, possibly because none lived longer than one year. The non-tumorous females lived to about the same age as the tumorous. One tumour was a large spindle-celled sarcoma, the remainder were papillomata. The difference in tumour incidence between the sexes was significant,[3] as was the increase in incidence in the injected females compared with the controls,[4] in spite of the earlier deaths of the former. The control males had a significantly higher incidence[5] than the injected males (3·3 per cent compared with nil), but some of the former lived to a maximum of 22 months, whereas the maximum for the latter was 12·5 months (Table I).

The numbers of M/CBA mice surviving to tumour age (18 months) and autopsied after December 1946 were very small. All 4 females but only one of the 7

* For [1] and following numbers see Appendix.

males had tumours, a significant difference[6] and possibly a real one, since all the non-tumour males lived beyond tumour age. Two females had large epitheliomata of the forestomach, the remaining tumours being papillomata. The tumour incidence was significantly higher in the injected females (100 per cent) than in the controls,[7] but in the injected males (14·3 per cent) the increase was not significant owing to the small numbers involved.

3. *Uninjected M/NBT F_1 and M/CBA F_1.*

These mice were the generation raised from inbreeding the injected pure strain animals. As in the pure strain controls, all the tumours were papillomata.

In M/NBT F_1 twice as many females as males were tumorous, a significant difference,[8] but one which could again be explained by the earlier deaths of the males. Although the non-tumorous males lived about as long as the tumour males, they died 2·6 months earlier than the non-tumorous females (Table I). Compared with the control NBT group, the F_1 tumour incidences of 29·4 per cent (females) and 15·2 per cent (males) were significantly higher.[9] This supports the earlier statement that the control tumour incidences might have been higher if the mice had lived longer, for the M/NBT F_1 non-tumour females lived on the average nearly as long as the F_1 tumour females and 2 months longer than the control NBT non-tumour females, thus giving greater opportunity for a true expression of the strain susceptibility. The F_1 non-tumour males also lived longer than the control non-tumour males, by 1·6 months.

Comparing the tumour incidences in the F_1 and the M/NBT groups, the incidence in the F_1 females was significantly less[10] although the F_1 females lived much longer; the incidence in the F_1 males should be compared not with that in the injected males (which were very few in number and died young) but with that in the injected females; these lived to the same age, yet the F_1 male tumour incidence was very much less.

In the M/CBA F_1 group, although there were fewer tumours in the males (again due to their earlier deaths), the difference in tumour incidence between the sexes was not significant and the incidences were also in agreement with those of the CBA controls, males and females. Compared with the injected M/CBA group, the F_1 females had a significantly lower tumour incidence,[11] but the incidence in the males, although less in F_1, was not significantly so on account of the small number of M/CBA males.

Since the apparent sex differences in tumour incidence were most probably due to different survival times, the average incidences for both sexes combined in each group were compared and the following results obtained. Tumour incidences in the NBT and uninjected M/NBT F_1 groups were higher than in the CBA and uninjected M/CBA F_1 groups, but in the injected mice of both strains they were the same, the carcinogen having obliterated the strain difference. Tumours were much more frequent in the two injected groups than in the controls and malignant tumours were found after injection but not in the controls. The uninjected descendants of each M/NBT and M/CBA group had lower incidences than their injected parents, but, whereas M/CBA F_1 had the same incidence as the CBA controls, the tumour incidence in M/NBT F_1 was higher than that in the NBT controls because the F_1 mice lived longer.

4. *Multiple papillomata.*

The effect of the methylcholanthrene was seen not only in the increased incidence of tumorous mice but also in the increased number (and size) of tumours per mouse, as shown in Table II.

NBT strain.—In the control NBT group the majority of tumorous mice each had only one small nodule in the forestomach, but 4 (11·4 per cent) females had 2 nodules each. Of the 17 tumorous M/NBT mice 7 (41·2 per cent) had 2 or more

TABLE II.—*The Incidence of Multiple Papillomata and of Gross Malignant Tumours of the Forestomach in Control and Injected Tumorous Mice Belonging to the Inbred NBT and CBA Parent Strains and the First Two Generations of Reciprocal Hybrids.*

Strain.	Generation.	Sex.	Number of tumour mice.	Mice with multiple papillomata. No.	Mice with multiple papillomata. Percentage.	Maximum counted number of papillomata per stomach.	Number of mice with gross malignant tumours.
NBT	—	F	28	4	14·3 } 11·4	2	0
		M	7	0	0·0 }	1	0
M/NBT	—	F	17	7	41·2 } 41·2	3*	1
		M	0	0	0·0 }	0	0
M/NBT	F_1	F	20	8	40·0 } 40·0	6*	0
		M	10	4	40·0 }	3	0
CBA	—	F	4	0	0·0 } 0·0	1	0
		M	0	0	0·0 }	0	0
M/CBA	—	F	4	0	0·0 } 0·0	1	2
		M	1	0	0·0 }	1	0
M/CBA	F_1	F	7	Not stated	—	—	0
		M	3	in records	—	—	0
NC	F_1	F	10	3	30·0 } 23·1	3	1
		M	3	0	0·0 }	1	0
NC	F_2	F	13	2	15·4 } 7·4	3*	0
		M	14	0	0·0 }	1	1
CN	F_1	F	10	2	20·0 } 20·0	2	0
		M	5	1	20·0 }	2	0
CN	F_2	F	9	2	22·2 } 11·8	2	0
		M	8	0	0·0 }	1	0
MNC	F_1	F	10	5	50·0 } 56·5	2*	2
		M	13	8	61·5 }	6	1
MNC	F_2	F	29	14	48·3 } 37·8	4*	3
		M	16	3	18·8 }	4	2
MCN	F_1	F	21	16	76·2 } 71·4	4*	4
		M	14	9	64·3 }	6*	0
MCN	F_2	F	35	21	60·0 } 55·3	7*	3
		M	12	5	41·7 }	3	1

* = Large number, uncounted, recorded as "multiple."

nodules each, one mouse having 3 and another so many that they were not counted. This increase is statistically significant.[12] There were as many tumour mice with multiple papillomata in the M/NBT F_1 group as in the injected group, the maximum number counted in one mouse being 6, while another had so many that they were recorded simply as "multiple". Although the F_1 mice were the longest-lived, this does not seem to be sufficient explanation of this result.

CBA strain—In the various CBA and M/CBA groups all the recorded occurrences were of solitary nodules. This is a further proof of the greater susceptibility of the NBT strain towards the formation of forestomach papillomata.

B. *Hybrid Strains—First Two Generations.*

1. *Spontaneous tumours in F_1 and F_2.*

In these two generations the numbers of mice available for analysis were considerably reduced by the necessity of omitting all dying before December 1946. Data for the incidence of forestomach tumours are given in Table III.

In each of the four groups, NC F_1, NC F_2, CN F_1 and CN F_2, the tumour incidences in the two sexes were in agreement. Where they appeared to differ widely, as in NC F_1 and CN F_1, the numbers were small, the males died 4 or 5 months earlier than the females, but the differences were not statistically significant. Where the males lived as long as the females, as in NC F_2 and CN F_2, the incidence in the males was slightly higher than in the females, but not significantly so. In all four groups the non-tumour mice lived as long as the tumour mice and the average age at death was about 2 years. Comparing the various groups the tum-

TABLE III.—*The Incidence of Forestomach Tumours in Control and Injected Mice of the First Two Generations of Reciprocal Hybrids.*

Strain and generation.	Sex.	Earliest tumour (months).	Effective number of mice.	Tumour mice. No.	Tumour mice. Percentage.	Tumour mice. Age at death (months). Range.	Tumour mice. Age at death (months). Average.	Non-tumour mice. Age at death (months). Range.	Non-tumour mice. Age at death (months). Average.
NC F_1	F	16·5	57	10	17·5 } 13·7	16·5–35·0	27·8 } 26·4	18·0–35·0	27·0 } 25·2
	M		38	3	7·9	21·5–23·0	22·0	16·5–31·5	22·8
NC F_2	F	16·5	103	13	12·6 } 13·2	19·0–29·0	24·5 } 23·7	16·5–31·5	24·1 } 23·6
	M		101	14	13·9	16·5–28·0	23·0	16·5–31·5	23·0
CN F_1	F	17·5	54	10	18·5 } 14·3	19·5–34·0	27·3 } 25·9	17·5–34·0	27·2 } 25·3
	M		51	5	9·8	17·5–28·0	23·2	17·5–31·5	23·9
CN F_2	F	18·0	117	9	7·7 } 8·2	18·0–28·5	23·9 } 23·7	18·0–32·5	27·6 } 24·3
	M		90	8	8·9	20·5–25·5	23·5	18·0–31·0	22·7
MNC F_1	F	9·0	17	10	58·8 } 57·5	14·0–26·0	18·8 } 18·5	9·0–31·0	15·9 } 16·4
	M		23	13	56·5	9·0–29·5	18·3	10·5–32·0	16·7
MNC F_2	F	6·0	94	29	30·9 } 23·6	6·0–24·5	16·0 } 15·5	6·0–26·5	11·6 } 11·6
	M		97	16	16·5	8·5–22·5	14·5	6·0–23·0	11·7
MCN F_1	F	8·0	27	21	77·8 } 63·6	9·0–24·0	18·2 } 18·5	8·0–28·5	20·0 } 16·8
	M		28	14	50·0	8·0–28·0	18·9	8·5–24·0	15·4
MCN F_2	F	8·0	74	35	47·3 } 35·1	8·0–28·0	15·6 } 15·0	8·0–28·5	15·4 } 13·9
	M		60	12	20·0	9·0–18·5	13·5	8·0–27·0	12·6

our incidences were all found to be in agreement, NC F_1 with NC F_2, CN F_1 with CN F_2, NC F_1 with CN F_1, and NC F_2 with CN F_2, the differences between percentages being less than twice the standard errors in all cases.

The tumour incidences in NC F_1 and CN F_1 were compared with those of NBT and also of M/NBT F_1, the latter on the assumption that it more truly represented the pure strain incidence owing to longer life. In none of the cases were the differences significant. The incidence in the F_1 hybrids was, therefore, that of the parent NBT strain—there was no sex-difference and no difference according to the direction of the cross.

As the mice of the early hybrid generations were long-lived, their tumours were found much later than those in the NBT mice and in this respect they resembled the other parent strain, the CBA. Although the tumours were of slow growth and could therefore have been present for a long time before death, yet mice dying in the younger age-groups were tumour-free, and it is believed that tumour development actually took place much later in the hybrids, being a function of the normal length of life of the mice.

With the exception of one gross epithelioma in an NC F_1 female and one in an NC F_2 male, the spontaneous tumours in the four groups were all papillomata.

2. *Induced tumours in F_1 and F_2.*

Data for tumour incidence in these generations of injected mice are given in Table III. The incidences were all much higher in the injected groups than in the controls. There was no sex-difference in tumour incidence in MNC F_1, but in the other three groups the incidences were significantly less in the males than in the females.[13] In MCN F_1 and MCN F_2 the non-tumour males died on the average several months earlier than the females, and in MNC F_2 the non-tumour mice of both sexes died at a very early average age.

Comparing MNC F_1 with MNC F_2 and MCN F_1 with MCN F_2, the drop in tumour incidence in the F_2 mice was significant in both cases,[14] and could be explained by the fact that the average age at death of injected F_2 mice was from 3 to nearly 5 months earlier than that of injected F_1 mice. There was no corresponding significant decline in the tumour incidence in the control F_2 mice, in which the decrease in the average age at death from F_1 to F_2 was much less.

Tumour incidences in MNC F_1 and MCN F_1 were in agreement, but the incidence of 23·6 per cent in MNC F_2 was significantly lower than that of 35·1 per cent in MCN F_2. Separate analysis showed that the difference lay in the females,[15] the incidences in the males being in agreement. The non-tumorous MNC F_2 females died 4 months earlier than the non-tumorous MCN F_2 females and also 4 months earlier than the tumorous MNC F_2 females; the tumour susceptibility of the MNC F_2 females was therefore probably not fully expressed.

Comparisons were made between the tumour incidences in the injected F_1 and F_2 hybrids and in the corresponding groups of controls. In every case the incidence in the injected mice was significantly higher.[16] As shown in Table III, tumours were found in injected mice from 7·5 to 8·5 months earlier than in the controls.

While the majority of tumours were papillomata, some of which showed signs of early malignancy, a number of large malignant growths were found in the injected mice (Table II). Thus there were 3 epitheliomata in MNC F_1 and 5 (one consisting of mixed epithelioma and sarcoma elements) in MNC F_2; the 4

gross tumours in MCN F_1 included one pure sarcoma, 1 mixed epithelioma-sarcoma and 2 pure epitheliomata, and there were 4 epitheliomata in MCN F_2.

3. *Multiple papillomata.*

As shown in Table II, the number of tumour mice bearing multiple papillomata was much lower in the control hybrids than in the injected hybrids. Widely though the percentages with multiple nodules vary between NC F_1 and F_2 and CN F_1 and F_2, none of the differences is significant and the percentages do not differ significantly from the proportion of NBT tumour mice bearing multiple papillomata. One NC F_1 mouse had 3 nodules, one in NC F_2 had so many that they were not counted, and another in F_2 had 3 nodules.

The contrast in the injected hybrids was striking. Except in MNC F_2, the percentage of tumour mice bearing 2 or more nodules was over 50 per cent in each group. The maximum number counted in one stomach was 7, but many were recorded as "multiple". In spite of quite wide fluctuations, the proportions with multiple nodules agreed amongst themselves in the different groups of injected hybrids (the difference being always less than twice the standard error) and all were significantly greater than in the corresponding control groups[17] (the difference being always greater than twice the standard error).

As stated in a previous communication (Miller and Pybus, 1954*b*), there was a possibility that some mice of the control F_1 groups (NC F_1 and CN F_1) might have become contaminated with methylcholanthrene either by contact with, or by licking injected mice of the same litters kept in the same boxes; the lung tumour incidences in these two groups were as high as in the injected F_1 mice. From the present data there is no positive evidence either from actual tumour incidence or from the percentages with multiple papillomata that such contamination, if it occurred, was sufficient to cause an increased incidence of forestomach tumours. The only possible evidence was, first, the case of the NC F_1 mouse bearing 3 papillomata (but one NC F_2 mouse had 3, and another "multiple", nodules and there was no possibility that any control F_2 animal had come in contact with the carcinogen) and, second, the finding of a large malignant epithelioma in an NC F_1 mouse (but again an F_2 animal was found to have a large malignant epithelioma, presumably spontaneous).

c. *Hybrid Strains—All Generations.*

1. *Spontaneous tumours, NC and CN groups.*

The data for spontaneous tumours in each generation of the NC and CN groups are given in Table IV, the figures for F_1 and F_2 being repeated from Table III for convenience of comparison. Comparing the same sexes, the total incidences for the 12 generations of 10·6 per cent for NC females and 8·5 per cent for CN females were in statistical agreement, as were the incidences of 5·5 per cent in both male groups.

In the NC group, although the incidences in the two sexes differed greatly in most generations, none of the differences was significant, but the totals of 10·6 per cent in females and 5·5 per cent in males in the 12 generations did differ significantly[18] (difference = 5·1, twice standard error = 3·3). In the CN group, the sex-differences in F_9 and F_{11} only were significant[19] (in F_9, difference = 10·0, twice standard error = 9·5; in F_{11}, difference = 7·1, twice standard error = 6·9),

TABLE IV.—*The Incidence of Spontaneous Tumours of the Forestomach in Each Generation of Control NC and CN Hybrids.*

Strain and generation.	Sex.	Effective number of mice.	Tumour mice. No.	Tumour mice. Percentage.	Tumour mice. Age at death (months). Range.	Tumour mice. Age at death (months). Average.	Non-tumour mice. Age at death (months). Range.	Non-tumour mice. Age at death (months). Average.
NC								
F_1	F	57	10	17·5	16·5–35·0	27·8	18·0–35·0	27·0
	M	38	3	7·9 } 13·7	21·5–23·0	22·0 } 26·4	16·5–31·5	22·8 } 25·2
F_2	F	103	13	12·6	19·0–29·0	24·5	16·5–31·5	24·1
	M	101	14	13·9 } 13·2	16·5–28·0	23·0 } 23·7	16·5–31·5	23·0 } 23·6
F_3	F	54	9	16·7	13·0–29·5	23·6	11·0–30·0	20·9
	M	43	1	2·3 } 10·3	19·5 —	19·5 } 23·2	8·0–31·0	15·9 } 18·4
F_4	F	60	4	6·7	19·5–27·0	24·6	9·0–29·0	22·5
	M	46	5	10·9 } 8·5	12·5–19·0	15·6 } 19·6	8·0–25·0	17·2 } 20·3
F_5	F	40	3	7·5	14·0–26·0	20·0	9·0–27·0	21·7
	M	46	0	0·0 } 3·5	— —	— } 20·0	9·0–24·0	17·4 } 19·3
F_6	F	45	3	6·7	16·5–21·5	21·6	8·0–25·0	19·1
	M	27	0	0·0 } 4·2	— —	— } 21·6	8·0–21·0	14·8 } 17·5
F_7	F	22	1	4·5	22·0 —	22·0	16·0–28·0	20·1
	M	46	2	4·4 } 4·4	9·0–18·0	13·5 } 16·3	8·0–23·0	16·2 } 17·4
F_8	F	48	7	14·6	14·5–26·5	21·2	9·0–27·0	19·5
	M	56	2	3·6 } 8·7	16·0–21·5	18·8 } 20·7	8·0–23·0	12·4 } 15·5
F_9	F	36	4	11·1	14·0–21·0	17·4	9·0–22·0	17·3
	M	36	1	2·8 } 6·9	8·0 —	8·0 } 15·5	8·0–20·0	13·5 } 15·3
F_{10}	F	31	1	3·2	8·0 —	8·0	8·0–29·0	17·7
	M	35	0	0·0 } 1·5	— —	— } 8·0	8·0–19·0	11·7 } 14·6
F_{11}	F	23	1	4·4	11·0 —	11·0	8·0–24·0	17·0
	M	9	0	0·0 } 3·1	— —	— } 11·0	8·0–15·0	10·9 } 14·9
F_{12}	F	28	2	7·1	12·5–14·0	13·3	9·0–23·0	14·8
	M	26	0	0·0 } 3·7	— —	— } 13·3	8·0–20·0	13·3 } 14·0
F_1–F_{12}	F	547	58	10·6	8·0–35·0	22·7	8·0–35·0	21·1
	M	509	28	5·5 } 8·1	8·0–28·0	19·9 } 21·8	8·0–31·5	16·8 } 18·9
CN								
F_1	F	54	10	18·5	19·5–34·0	27·3	17·5–34·0	27·2
	M	51	5	9·8 } 14·3	17·5–28·0	23·2 } 25·9	17·5–31·5	23·9 } 25·3
F_2	F	117	9	7·7	18·0–28·5	23·9	18·0–32·5	27·6
	M	90	8	8·9 } 8·2	20·5–25·5	23·5 } 23·7	18·0–31·0	22·7 } 24·3
F_3	F	40	7	17·5	10·0–28·5	22·5	10·0–32·0	23·2
	M	33	4	12·1 } 14·3	12·5–23·0	17·4 } 20·6	9·0–28·0	18·6 } 20·6
F_4	F	35	4	11·4	22·0–29·5	25·2	9·0–29·0	20·4
	M	36	6	16·7 } 14·1	11·0–25·0	20·2 } 22·4	8·0–28·0	16·1 } 18·3
F_5	F	28	3	10·7	8·0–26·5	19·0	8·0–33·0	22·4
	M	23	0	0·0 } 5·9	— —	— } 19·0	8·0–23·0	16·2 } 19·4
F_6	F	25	2	8·0	21·0–26·5	23·8	11·0–27·0	19·9
	M	13	0	0·0 } 5·3	— —	— } 23·8	8·0–14·0	10·8 } 16·6

TABLE IV—*cont.*

Strain and generation.	Sex.	Effective number of mice.	Tumour mice. No.	Tumour mice. Percentage.	Tumour mice. Age at death (months). Range.	Tumour mice. Age at death (months). Average.	Non-tumour mice. Age at death (months). Range.	Non-tumour mice. Age at death (months). Average.
F_7	F	24	1	4·2 } 1·9	20·0 —	20·0 } 20·0	8·0–30·0	17·6 } 14·9
	M	29	0	0·0 }	— —	— }	8·0–24·0	12·7 }
F_8	F	38	4	10·5 } 7·8	20·5–27·5	24·6 } 19·9	10·0–28·0	20·6 } 16·3
	M	39	2	5·1 }	9·5–11·5	10·5 }	8·0–23·0	12·3 }
F_9	F	40	4	10·0 } 6·7	25·0–30·0	26·6 } 26·6	9·0–32·0	20·3 } 16·8
	M	20	0	0·0 }	— —	— }	8·0–14·0	10·7 }
F_{10}	F	53	2	3·8 } 3·4	16·0–22·0	19·0 } 17·2	8·0–28·0	17·9 } 15·6
	M	36	1	2·8 }	13·5 —	13·5 }	8·0–18·0	12·4 }
F_{11}	F	56	4	7·1 } 4·8	10·0–20·0	13·8 } 13·8	8·0–24·0	15·6 } 13·5
	M	28	0	0·0 }	— —	— }	8·0–15·0	9·5 }
F_{12}	F	116	3	2·6 } 2·4	18·5–24·5	21·7 } 18·6	8·0–29·0	16·3 } 14·8
	M	131	3	2·3 }	13·0–27·0	15·5 }	8·0–19·0	13·4 }
F_1–F_{12}	F	620	53	8·5 } 7·1	8·0–34·0	23·3 } 22·1	8·0–34·0	19·3 } 17·3
	M	529	29	5·5 }	9·5–28·0	19·9 }	8·0–31·5	15·0 }

and the difference between the totals for the 12 generations of 8·5 per cent in females and 5·5 per cent in males was barely significant (difference = 3·0, twice standard error = 2·99). In both groups the males died several months earlier than the females, and this is believed to account for the difference in tumour incidences in the two sexes.

In the later generations of both groups there was a tendency towards earlier death in both sexes and this is reflected in the trend towards lower tumour incidences in later generations, this trend being noticeable in spite of the wide fluctuations from generation to generation. While there might be a genetical basis for the diminishing incidences, it was not possible to discern this in the case of a tumour essentially of old age and with such a low incidence in younger mice that often there was only a solitary occurrence, or even none, in a small generation in which many mice died young.

Spontaneous gross malignant tumours were rare, none being seen in the CN group, and (in addition to those mentioned earlier) only 1 in an NC F_3 female and 2 in NC F_8 females, all squamous epitheliomata, giving a total in NC of 4 gross tumours in females and 1 in a male.

Multiple papillomata. All the other spontaneous tumours in these groups were papillomata, the great majority being solitary. In NC (F_3 to F_{12}) only 2 out of 5 F_3 tumour males and 1 out of 7 F_8 tumour females each had 2 papillomata; including F_1 and F_2 (Table II) this gave a total of 6 cases of multiple nodules in 58 tumour females (10·4 per cent) and 2 in 28 tumour males (7·1 per cent). This difference was not significant. In the control CN mice (F_3 to F_{12}) 1 F_4 female had 2 papillomata, the remainder being solitary. With F_1 and F_2 (Table II) this gave a total of 5 cases of multiple nodules in 53 tumour females (9·4 per cent) and 1 in 29 males (3·4 per cent), again an insignificant difference. The

incidences of multiple nodules in the two control groups were in agreement for both males and females.

2. *Induced tumours, MNC and MCN groups.*

Table V presents the data for the tumour incidence in each generation of the injected hybrids, the figures for F_1 and F_2 being repeated from Table III for comparison. There was wide variation in tumour incidence between the sexes. In MNC F_1, F_3, F_6 and F_9 this difference was not significant, but in the remaining generations and in the totals for all 10 generations in this group the differences were significant.[20] In the MCN group the sex-difference in tumour incidence was significant in F_1, F_2 and F_3 and in the total for the 10 injected generations.[21] In both injected groups the non-tumour mice died young, their average age being several months less than the average tumour age, but the difference in age at death between males and females was much less constant than in the control NC and CN hybrids.

In both groups there was a decrease in tumour incidence in the later generations compared especially with F_1 and F_2, and this was paralleled by a decrease in the average survival age of the mice. The very low incidences in the last 5 generations of MCN were due to the early deaths, very few mice living to the age of 18 months.

The total tumour incidences in the two injected groups were in agreement, females with females (27·7 per cent in MNC, 27·2 per cent in MCN) and males with males (12·5 per cent in MNC, 14·1 per cent in MCN). There was also very little difference between the average ages at death of tumour mice in the two groups, and also of non-tumour mice.

Tumour incidences were significantly higher in the MNC and MCN hybrids than in the control NC and CN groups,[22] by as much as two to three times, and tumours were found much earlier in the injected groups—by about 8 months.

Multiple papillomata.—Table VI shows the incidence of multiple papillomata in the injected MNC and MCN generations. Nearly 50 per cent of the injected tumorous mice had 2 or more nodules each, and whereas in the controls (where less than 10 per cent of tumorous mice bore 2 or more nodules) the maximum number counted in one individual was 3, in the injected mice the maximum number counted was 9, and there were many cases recorded simply as "multiple", the papillomata being so numerous.

The proportions of tumour-bearing injected mice in the two groups which had multiple papillomata were in agreement (48·8 per cent of 168 MNC tumour females and 46·0 per cent of 124 MCN tumour females; 37·1 per cent of 89 MNC tumour males and 44·6 per cent of 65 MCN tumour males) and there was no significant sex-difference.

There were 26 cases of gross epitheliomata and sarcomata in the MNC mice and 16 cases in the MCN mice.

3. *The uninjected MNC and MCN groups.*

Table VII gives the incidence of spontaneous tumours in these descendants of injected mice. In the uninjected MNC group the differences between the sexes were not significant except in F_{12} (a large generation) and in the totals for F_5 to F_{12}.[23] Tumour incidence fluctuated from generation to generation, and the totals

TABLE V.—*The Incidence of Forestomach Tumours in Each Generation of Methylcholanthrene-injected MNC and MCN Hybrids.*

Strain and generation.	Sex.	Effective number of mice.	Tumour mice. No.	Tumour mice. Percentage.	Tumour mice. Age at death (months). Range.	Tumour mice. Age at death (months). Average.	Non-tumour mice. Age at death (months). Range.	Non-tumour mice. Age at death (months). Average.
MNC								
F_1	F	17	10	58·8	14·0–26·0	18·8	9·0–31·0	15·9
	M	23	13	56·5	9·0–29·5	18·3	10·5–32·0	16·7
				57·5		18·5		16·4
F_2	F	94	29	30·9	6·0–24·5	16·0	6·0–26·5	11·6
	M	97	16	16·5	8·5–22·5	14·5	6·0–23·0	11·7
				23·6		15·5		11·6
F_3	F	33	9	27·3	5·0–23·0	13·8	5·0–17·0	9·5
	M	36	6	16·7	6·5–11·5	9·3	5·0–13·0	7·8
				21·7		12·0		8·6
F_4	F	107	50	46·7	5·5–26·5	13·4	4·0–29·0	9·4
	M	110	30	27·3	4·0–19·5	9·7	4·0–24·0	7·9
				36·9		12·0		8·6
F_5	F	60	23	36·3	5·0–24·5	10·4	4·0–23·5	7·9
	M	88	7	8·0	5·0–20·5	9·2	4·0–18·0	7·4
				20·3		10·1		7·6
F_6	F	49	9	18·4	6·0–23·5	11·4	4·0–23·5	8·8
	M	51	6	11·8	7·0–16·5	10·1	4·0–17·0	7·4
				15·0		10·9		7·7
F_7	F	84	14	16·7	7·0–27·0	16·4	4·0–28·0	9·0
	M	96	3	3·1	8·5–16·5	11·5	4·0–20·0	8·5
				9·4		15·6		8·7
F_8	F	31	8	25·8	5·0–25·0	12·8	4·0–24·0	10·0
	M	50	2	4·0	6·0–20·0	13·0	5·0–17·0	7·4
				12·4		12·9		8·2
F_9	F	62	5	8·1	14·5–22·0	18·4	4·0–23·0	8·7
	M	79	2	2·5	6·5–13·5	10·0	4·0–22·0	9·4
				5·0		16·0		9·1
F_{10}	F	69	11	15·9	11·5–27·0	17·6	4·0–22·0	7·9
	M	80	4	5·0	16·0–19·0	17·6	4·0–25·0	9·6
				10·1		17·6		8·9
F_1–F_{10}	F	606	168	27·7	5·0–27·0	14·3	4·0–31·0	9·3
	M	710	89	12·5	4·0–29·5	12·3	4·0–32·0	8·9
				19·5		13·6		9·1
MCN								
F_1	F	27	21	77·8	9·0–24·0	18·2	8·0–28·5	20·0
	M	28	14	50·0	8·0–28·0	18·9	8·5–24·0	15·4
				63·6		18·5		16·8
F_2	F	74	35	47·3	8·0–28·0	15·6	8·0–28·5	15·4
	M	60	12	20·0	9·0–18·5	13·5	8·0–27·0	12·6
				35·1		15·0		13·9
F_3	F	45	23	51·1	5·5–27·0	17·6	6·0–26·0	12·4
	M	46	6	13·0	9·0–19·5	13·5	5·0–28·0	11·7
				31·9		16·8		11·9
F_4	F	59	18	30·5	5·5–21·5	10·5	4·0–26·0	10·4
	M	58	16	27·6	6·0–20·0	10·1	4·0–24·0	8·5
				29·1		10·3		9·4
F_5	F	58	18	31·0	7·5–24·5	13·0	4·0–23·0	9·7
	M	66	13	19·7	4·0–19·5	10·5	4·0–26·0	9·7
				25·0		12·0		9·7
F_6	F	12	0	0·0	—	—	4·0–20·0	12·3
	M	13	0	0·0	—	—	4·0– 8·0	5·5
				0·0		—		8·7
F_7	F	25	0	0·0	—	—	5·0–17·0	9·3
	M	25	0	0·0	—	—	4·0–16·0	8·4
				0·0		—		8·9
F_8	F	53	4	7·6	7·0–16·5	12·1	4·0–18·0	8·2
	M	70	2	2·9	7·5–11·5	9·5	4·0–17·0	7·9
				4·9		11·2		8·0
F_9	F	39	2	5·1	12·0–18·5	15·3	4·0–17·0	9·4
	M	44	0	0·0	—	—	4·0–16·0	7·4
				2·4		15·3		8·3
F_{10}	F	64	3	4·7	12·5–15·0	14·2	4·0–17·0	8·6
	M	50	2	4·0	9·5–12·5	11·0	4·0–15·0	8·6
				4·4		12·9		8·6
F_1–F_{10}	F	456	124	27·2	5·5–28·0	15·1	4·0–28·5	10·4
	M	460	65	14·1	4·0–28·0	13·0	4·0–28·0	9·4
				20·6		14·4		9·9

TABLE VI.—*Incidences of Multiple Papillomata and of Gross Malignant Tumours of the Forestomach in Each Generation of Methylcholanthrene-injected MNC and MCN Hybrids.*

Strain and generation.	Sex.	Number of tumour mice.	Mice with multiple papillomata.			Maximum counted number of papillomata per stomach.	Number of mice with gross malignant tumours.
			No.	Percentage.			
F_1	F	10	5	50·0	56·5	2*	2
	M	13	8	61·5		6	1
F_2	F	29	14	48·3	37·8	4*	3
	M	16	3	18·8		4	2
F_3	F	9	8	88·9	73·3	5	0
	M	6	3	50·0		2*	0
F_4	F	50	30	60·0	57·5	9*	7
	M	30	16	53·3		3*	4
F_5	F	23	9	39·1	36·7	5	1
	M	7	2	28·6		2	0
F_6	F	9	6	66·7	40·0	4	0
	M	6	0	0·0		1	0
F_7	F	14	4	28·6	23·5	3	2
	M	3	0	0·0		1	0
F_8	F	8	2	25·0	30·0	2	0
	M	2	1	50·0		5	0
F_9	F	5	1	20·0	14·3	2	1
	M	2	0	0·0		1	1
F_{10}	F	11	3	27·3	20·0	3	2
	M	4	0	0·0		1	0
F_1–F_{10}	F	168	82	48·8	44·7	9*	18
	M	89	33	37·1		6*	8
MCN							
F_1	F	21	16	76·2	71·4	4*	4
	M	14	9	64·3		6*	0
F_2	F	35	21	60·0	55·3	7*	3
	M	12	5	41·7		3	1
F_3	F	23	6	26·1	27·6	4*	6
	M	6	2	33·3		4	1
F_4	F	18	8	44·4	50·0	5	0
	M	16	9	56·3		5*	1
F_5	F	18	6	33·3	32·3	4	0
	M	13	4	30·8		6	0
F_6	F	0	0	0·0	0·0	0	0
	M	0	0	0·0		0	0
F_7	F	0	0	0·0	0·0	0	0
	M	0	0	0·0		0	0
F_8	F	4	0	0·0	0·0	1	0
	M	2	0	0·0		1	0

TABLE VI—*cont.*

Strain and generation. MNC	Sex.	Number of tumour mice.	Mice with multiple papillomata. No.	Mice with multiple papillomata. Percentage.	Maximum counted number of papillomata per stomach.	Number of mice with gross malignant tumours.
F_9	F	2	0	0·0	1	0
	M	0	0	0·0 (0·0)	0	0
F_{10}	F	3	0	0·0	1	0
	M	2	0	0·0 (0·0)	1	0
F_1–F_{10}	F	124	57	46·0	7*	13
	M	65	29	44·6 (45·5)	6*	3

* = Large number, uncounted, recorded as " multiple."

of 9·3 per cent for females and 4·4 per cent for males were in agreement with the control NC values of 10·6 per cent and 5·5 per cent respectively (Table IV). The ages at death of the tumorous and non-tumorous uninjected MNC mice were also comparable with the control values.

The uninjected MCN mice died young and the tumour incidences (1·9 per cent in females and 1·5 per cent in males) were low. The sex-differences were nowhere significant. The tumour incidences were significantly less than those in the uninjected MNC mice, and also significantly less than those in the CN controls.[24]

There was no evidence that the increased incidence and earlier appearance of forestomach tumours in the injected mice were characteristics continued in their uninjected descendants. Especially in F_{12} of the uninjected MNC hybrids, a very large generation and one that was preceded by 10 generations of injected mice and one of uninjected, the tumour incidences of 10·8 per cent in the females and 5·0 per cent in the males were in agreement with the total incidences of 10·6 per cent and 5·5 per cent respectively in the NC controls.

Multiple papillomata.—Table VIII shows the incidence of multiple papillomata in the 8 generations of uninjected MNC mice. The maximum individual number was 3 in an F_{11} mouse. The total incidences of 7·7 per cent in 78 tumorous females and 2·1 per cent in 48 tumour males (not a significant difference) did not differ significantly from the corresponding values in the control NC hybrids. There were 6 cases of malignant epitheliomata in the uninjected MNC group, 4 of which were quite small tumours and none was so florid as those found in the injected mice.

Of the few tumorous mice in the uninjected MCN group none had multiple papillomata, but 2 malignant epitheliomata, both rather small, were found in F_5.

D. *Malignant Tumours.*

Bagshaw and Strong (1950) found that malignant epidermoid carcinomata of the forestomach appeared earlier than benign papillomata. The ages at death of all mice in the present experiment bearing malignant forestomach tumours

TABLE VII.—*The Incidence of Forestomach Tumours in the Uninjected Offspring of Injected Hybrids.*

Strain and generation.	Sex.	Effective number of mice.	Tumour mice. No.	Tumour mice. Percentage.	Tumour mice. Age at death (months). Range.	Tumour mice. Age at death (months). Average.	Non-tumour mice. Age at death (months). Range.	Non-tumour mice. Age at death (months). Average.
MNC (uninj.)								
F_5	F	27	0	0·0 } 1·6	— —	— } 16·5	6·0–27·0	18·8 } 16·9
	M	35	1	2·9 }	16·5 —	16·5 }	7·0–29·0	15·5 }
F_6	F	100	14	14·0 } 9·9	19·0–28·0	23·4 } 22·7	6·0–29·0	20·9 } 19·4
	M	112	7	6·3 }	15·5–23·5	21·3 }	6·0–30·0	18·1 }
F_7	F	77	4	5·2 } 5·9	19·0–27·0	23·0 } 21·2	6·0–29·0	21·0 } 18·5
	M	77	5	6·5 }	15·5–27·0	19·8 }	6·0–26·0	15·9 }
F_8	F	42	3	7·2 } 4·5	24·0–27·0	26·0 } 25·8	9·0–28·0	21·5 } 17·7
	M	46	1	2·2 }	25·0 —	25·0 }	6·0–26·0	14·3 }
F_9	F	34	2	5·9 } 2·6	20·0–25·5	22·8 } 22·8	6·0–28·0	20·2 } 18·3
	M	44	0	0·0 }	— —	— }	8·0–26·0	16·8 }
F_{10}	F	80	9	11·3 } 8·3	15·0–26·5	20·9 } 21·1	7·0–28·0	19·9 } 18·6
	M	77	4	5·2 }	18·5–26·0	21·6 }	6·0–27·0	17·4 }
F_{11}	F	147	10	6·8 } 4·5	6·0–28·5	19·2 } 19·7	6·0–26·0	17·8 } 17·1
	M	209	6	2·9 }	14·5–23·5	20·5 }	6·0–25·0	16·6 }
F_{12}	F	334	36	10·8 } 7·5	12·0–28·5	22·3 } 21·2	6·0–28·0	19·6 } 18·7
	M	440	22	5·0 }	14·5–23·5	19·5 }	6·0–28·0	18·1 }
F_5–F_{12}	F	841	78	9·3 } 6·6	6·0–28·5	22·1 } 21·4	6·0–29·0	17·9 } 17·1
	M	1040	46	4·4 }	14·5–27·0	20·2 }	6·0–30·0	16·5 }
MCN (uninj.)								
F_5	F	38	1	2·6 } 4·6	23·5 —	23·5 } 19·9	11·0–25·0	19·3 } 18·7
	M	49	3	6·1 }	10·0–25·0	18·7 }	11·0–25·0	18·3 }
F_6	F	3	0	0·0 } 0·0	— —	— } —	16·0–20·0	18·0 } 17·8
	M	2	0	0·0 }	— —	— }	14·0–21·0	17·5 }
F_7	F	17	0	0·0 } 0·0	— —	— } —	10·0–18·0	14·8 } 14·4
	M	24	0	0·0 }	— —	— }	12·0–17·0	14·2 }
F_8	F	31	1	3·2 } 1·5	16·0 —	16·0 } 16·0	11·0–19·0	15·4 } 14·2
	M	38	0	0·0 }	— —	— }	10·0–17·0	13·2 }
F_9	F	40	1	2·5 } 3·9	18·0 —	18·0 } 16·2	11·0–21·0	16·3 } 15·6
	M	36	2	5·6 }	14·5–16·0	15·3 }	10·0–22·0	14·8 }
F_{10}	F	46	1	2·2 } 1·9	18·5 —	18·5 } 18·5	10·0–19·0	14·6 } 13·9
	M	38	0	0·0 }	— —	— }	10·0–20·0	13·1 }
F_{11}	F	90	2	2·2 } 1·1	14·0–15·5	14·8 } 14·8	10·0–24·0	13·7 } 13·2
	M	85	0	0·0 }	— —	— }	10·0–21·0	12·8 }
F_{12}	F	114	1	0·9 } 0·8	17·0 —	17·0 } 15·5	10·0–20·0	13·6 } 13·3
	M	125	1	0·8 }	14·0 —	14·0 }	10·0–17·0	13·1 }
F_5–F_{12}	F	379	7	1·9 } 1·7	14·5–23·5	17·5 } 17·2	10·0–25·0	14·8 } 14·4
	M	397	6	1·5 }	10·0–25·0	16·8 }	10·0–25·0	13·9 }

TABLE VIII.—*Incidences of Multiple Papillomata and of Gross Malignant Tumours of the Forestomach in Each Generation of Uninjected Descendants of Injected MNC Mice.*

Strain and generation.	Sex.	Number of tumour mice.	Mice with multiple papillomata. No.	Mice with multiple papillomata. Percentage.		Maximum counted number of papillomata per stomach.	Number of mice with gross malignant tumours
MNC (uninjected)							
F_5	F	0	0	0·0	0·0	0	0
	M	1	0	0·0		1	0
F_6	F	14	0	0·0	0·0	1	3
	M	7	0	0·0		1	0
F_7	F	4	0	0·0	11·1	1	0
	M	5	1	20·0		2	0
F_8	F	3	1	33·3	25·0	2	0
	M	1	0	0·0		1	0
F_9	F	2	0	0·0	0·0	1	0
	M	0	0	0·0		0	0
F_{10}	F	9	2	22·2	15·4	2	0
	M	4	0	0·0		1	0
F_{11}	F	10	1	10·0	6·3	3	1
	M	6	0	0·0		1	0
F_{12}	F	36	2	5·6	3·4	2	0
	M	22	0	0·0		1	2
F_5–F_{12}	F	78	6	7·7	5·6	3	4
	M	46	1	2·2		2	2

are shown in Table IX, for comparison with the average ages of discovery of all forestomach tumours, benign and malignant, given in Tables I, IV, V and VII. With a few exceptions, the ages of discovery of spontaneous and induced malignant gross tumours were slightly greater than the corresponding average ages for all tumours. It must again be pointed out that " tumour age " in the present experiment was the age at which the tumour was discovered, that a tumour might have been present in the animal for some time before death, and that a gross malignant tumour could be the cause of death. Stewart and Lorenz (1949) make a sharp distinction between benign papillomata and gross carcinomata, stating that the former do not necessarily lead to the latter; and in the present material there were instances where both types were present in the same stomach.

DISCUSSION.

Very few references have been found in the literature to the presence of spontaneous benign papillomata of the forestomach in mice. Stewart and Andervont (1938) quote Bonné (1927) who found 2 mice with stomach papillomata in a total of 146 controls, and Stewart and Lorenz (1949) found solitary papillomata in control mice receiving mineral oil. Peacock, Beck and Chalmers (1953) saw no spontaneous papillomata in 86 control mice, but found 3 in 99 mice treated with

TABLE IX.—*The Ages at Death of Mice Bearing Malignant Forestomach Tumours.*

Strain and generation.	Individual ages (months).		Average ages.		Group averages.	
	Females.	Males.	Females.	Males.	Females.	Males.
M/NBT	13·5	—	13·5	—	13·5	—
M/CBA	20·0, 27·0	—	23·5	—	23·5	—
NC						
F_1	29·0	—	29·0	—		
F_2	—	16·5	—	16·5		
F_3	25·0	—	25·0	—	24·5	16·5
F_4	17·5, 26·5	—	22·0	—		
Uninj. MNC						
F_6	19·0, 23·5, 26·5	—	23·0	—		
F_{11}	20·0	—	20·0	—	22·3	23·3
F_{12}	—	23·0, 23·5	—	23·3		
Uninj. MCN						
F_5	23·5	10·0	23·5	10·0	23·5	10·0
Total "spontaneous" average age (hybrids).					23·4	18·3
MNC						
F_1	20·5, 21·0	9·0	20·8	9·0		
F_2	20·0, 19·0, 24·5	14·5, 10·5	21·2	12·5		
F_3	19·5, 5·0	—	12·5	—		
F_4	20·0, 21·5, 20·0, 10·0, 19·0, 14·0, 12·0	13·5, 4·0, 13·0 19·5	16·6 —	12·5 —		
F_5	19·5, 14·0	—	16·8	—	17·1	12·2
F_7	14·0, 16·0	—	15·0	—		
F_8	18·0	—	18·0	—		
F_9	15·0	13·5	15·0	13·5		
F_{10}	12·0, 21·0	—	16·5	—		
MCN						
F_1	21·0, 23·0, 11·5, 14·5	—	17·5	—		
F_2	17·5, 16·0, 25·5	16·5	19·7	16·5		
F_3	26·5, 21·5, 26·5, 23·0 22·5, 18·5	17·0	23·1	17·0	19·8	12·1
F_4	9·0	7·5, 7·5	9·0	7·5		
Total "induced" average age (hybrids)					18·1	12·2

control solvents. They are tumours of infrequent occurrence which could readily escape observation unless the stomachs of all mice were submitted to routine examination at autopsy. Very occasional examples have been seen in recent years in this laboratory in the A and GFF strains. In the present case it happened that 2 inbred strains, NBT and CBA, were crossed, in which the incidences of this tumour, although small, differed significantly. In the reciprocal hybrids the incidence did not vary with the direction of the cross and was similar to that of the NBT strain—the parent strain with the higher incidence. The tumour occurs in middle and old age, and the observed incidence was therefore very closely connected with the average age of the mice at death, relative to the normal length of life of the strain. Thus the incidence in males was usually less than that in

females, because the males tended to die sooner ; where they lived as long as the females the tumour-incidence was the same in both sexes. It is therefore believed that this tumour-incidence shows no real sex-difference, but because of the apparent sex-difference it has been thought more accurate to give the incidences in the two sexes separately and, in comparing two groups of mice, to compare females with females and males with males.

Although spontaneous malignant tumours of the forestomach were not seen in the two parent strains they have been known to occur occasionally in mice (Slye, Holmes and Wells, 1917 ; Wells, Slye and Holmes, 1938 ; Collins, Gardner and Strong, 1943 ; Jackson Memorial Laboratory, 1941). A few were found in the hybrids in the present experiment in mice which at no time had been, or could have been, exposed to methylcholanthrene (unless, as Beck (1952) suggested for benzpyrene, there had been some slight contamination of boxes with the carcinogen from previous occupants which had been injected) ; malignant tumours found in the F_1 mice must of course be suspect.

In spite of the rarity of the spontaneous gastric neoplasm, benign and malignant forestomach tumours have been produced experimentally in mice since 1927. Historical surveys of experimentally induced gastric tumours, up to 1940, have been given by Stewart (1940) and by Klein and Palmer (1941). Since then, Lorenz and Stewart (1940, 1948), Stewart (1941) and Stewart and Lorenz (1942, 1949) have published several reports on the induction of forestomach tumours by oral administration of carcinogens as well as by direct injection (the method by which they produced the first experimentally-induced glandular gastric carcinoma). Collins, Gardner and Strong (1943) induced benign and malignant forestomach tumours by intra-vaginal instillation and by oral administration of carcinogens, while Strong (1945) and Bagshaw and Strong (1950) obtained similar tumours in mice exposed to the carcinogen by remote injection, as in the present work. Although Gardner (1941) and Collins, Gardner and Strong (1943) believed that the tumours developed in those mice receiving the carcinogen by the vaginal route because the mice licked themselves and each other, thus swallowing some of the carcinogen which had oozed out, they obtained fewer tumours in this way than by direct oral administration ; and Bagshaw and Strong (1950) did not think that the small amount obtained by licking could account for the tumours produced in their experiment.

It has already been shown (Miller and Pybus, 1954*b*) that the control F_1 mice in the present experiment apparently obtained enough methylcholanthrene by licking their injected litter-mates to increase the lung tumour incidence to that in injected mice. Evidently the quantity of carcinogen thus received was not sufficient to raise the incidence of forestomach papillomata in the control F_1 mice above normal. The stomach tumour incidence in the injected mice was very much greater than that in the controls and the tumours appeared many months earlier, while, in addition to the increased proportion of tumorous mice, the number of papillomata per stomach was also greatly increased, and epidermoid carcinomata as well as sarcomata and mixed tumours were present in much greater numbers. There can be no doubt that the increase was due to the methylcholanthrene, but the effect in the present material did not persist in the uninjected descendants of the injected mice ; these uninjected mice had a tumour incidence similar to that of the controls, even when they were descended from 10 generations of injected animals.

The incidence of forestomach tumours in mice treated with carcinogens has been found to depend on the strain of mice. Strain differences were found by Gardner (1941), Collins, Gardner and Strong (1943) and by Lorenz and Stewart (1940, 1948), while Strong, Collins and Durand (1943) and Bagshaw and Strong (1950) found a difference between two sublines, one of which produced forestomach tumours and the other glandular gastric tumours. Susceptibility has been found to depend also on the carcinogen used as well as on its mode of administration. In the present work two inbred strains of mice with different incidences of spontaneous forestomach tumours were injected, but their susceptibilities to methylcholanthrene were the same; admittedly the numbers of injected mice surviving to tumour age were small, the carcinogen used was one of the more potent (Klein and Palmer, 1941; Lorenz and Stewart, 1948), and the dose was large.

SUMMARY.

1. Two inbred strains of mice, CBA and NBT, which differed in the incidence of spontaneous papillomata of the forestomach, were crossed reciprocally. A number of mice from the parent strains and from ten inbred generations of the hybrids each received one subcutaneous injection of 1·0 mg. methylcholanthrene at the age of two months.

2. The incidence of spontaneous forestomach papillomata in the hybrids did not vary with the direction of the cross and was the same as that of the more susceptible parent strain, the NBT, but the tumours appeared much later, as in the CBA strain. There was no real sex-difference in incidence, but only an apparent one caused by the earlier deaths of the males.

3. A few epidermoid carcinomata of the forestomach, presumed to be spontaneous, were seen in hybrids not exposed to the carcinogen, but none occurred in the parent strains.

4. In the injected groups the incidence of tumorous mice was greatly increased, the number of papillomata per stomach was also increased, and the tumours were produced many months earlier than in the controls. The incidence of malignant tumours of the forestomach (epidermoid carcinomata, sarcomata, and mixed carcino-sarcomata) was increased.

5. Injected members of the two inbred strains and the F_1 hybrids all had the same incidence of induced forestomach papillomata. In the later hybrid generations the induced tumour incidence fell as fewer mice survived to old age.

6. There was no evidence that the increased tumour incidence in the injected mice was maintained in their uninjected descendants, even after ten injected generations. The incidence in the uninjected descendants was similar to that in the controls.

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Appendix

Statistics of significance for *significant* differences only.

Index No.	Groups compared.	Difference.	Twice standard error.
1	NBT ♀ *v.* ♂	8·5	4·8
2	NBT ♀ *v.* CBA ♀	9·3	4·7
	NBT ♂ *v.* CBA ♂	3·3	2·4
	NBT ♀ + ♂ *v.* CBA ♀ + ♂	6·3	5·1
3	M/NBT ♀ *v.* ♂	77·3	17·9
4	M/NBT ♀ *v.* NBT ♀	65·6	18·3
5	M/NBT ♂ *v.* NBT ♂	3·3	2·4
6	M/CBA ♀ *v.* ♂	85·7	26·5
7	M/CBA ♀ *v.* CBA ♀	97·7	2·0
8	M/NBT F_1 ♀ *v.* ♂	14·26	14·16
9	M/NBT F_1 ♀ *v.* NBT ♀	17·7	11·8
	M/NBT F_1 ♂ *v.* NBT ♂	11·9	9·2
10	M/NBT F_1 ♀ *v.* M/NBT ♀	47·9	21·9
11	M/CBA F_1 ♀ *v.* M/CBA ♀	95·7	3·2
12	M/NBT *v.* NBT	29·8	13·1
13	MNC F_2 ♀ *v.* ♂	14·4	12·2
	MCN F_1 ♀ *v.* ♂	27·8	24·8
	MCN F_2 ♀ *v.* ♂	27·3	15·5
14	MNC F_1 ♀ *v.* MNC F_2 ♀ (♂ significant by inspection)	27·9	19·6
	MCN F_1 ♀ *v.* MCN F_2 ♀	30·5	19·8
	MCN F_1 ♂ *v.* MCN F_2 ♂	30·0	21·5
15	MNC F_2 ♀ + ♂ *v.* MCN F_2 ♀ + ♂	11·5	10·3
	MNC F_2 ♀ *v.* MCN F_2 ♀	16·4	15·0
16	MNC F_1 *v.* NC F_1	43·8	17·2
	MNC F_2 *v.* NC F_2	10·4	7·8
	MCN F_1 *v.* CN F_1	39·3	14·7
	MCN F_2 *v.* CN F_2	26·9	9·1
17	MNC F_1 *v.* NC F_1	33·1	31·2
	MNC F_2 *v.* NC F_2	30·4	17·6
	MCN F_1 *v.* CN F_1	51·4	25·7
	MCN F_2 *v.* CN F_2	43·5	21·3
18	NC ♀ *v.* ♂ (total)	5·1	3·3
19	CN F_9 ♀ *v.* ♂	10·0	9·5
	CN F_{11} ♀ *v.* ♂	7·1	6·9
	CN ♀ *v.* ♂ (total)	3·0	2·99
20	MNC F_4 ♀ *v.* ♂	19·4	12·9
	MNC F_5 ♀ *v.* ♂	28·3	13·7
	MNC F_7 ♀ *v.* ♂	13·6	8·9
	MNC F_8 ♀ *v.* ♂	21·8	16·7
	MNC F_{10} ♀ *v.* ♂	10·9	10·1
	MNC ♀ *v.* ♂ (total)	15·2	4·4
21	MCN F_3 ♀ *v.* ♂	38·1	17·9
	MCN ♀ *v.* ♂ (total)	13·1	5·3
22	MNC ♀ *v.* NC ♀	17·1	4·5
	MNC ♂ *v.* NC ♂	7·0	3·2
	MCN ♀ *v.* CN ♀	18·7	4·7
	MCN ♂ *v.* CN ♂	8·6	3·8
23	uninj. MNC F_2 ♀ *v.* ♂	5·8	3·98
	uninj. MNC ♀ *v.* ♂ (total)	4·9	2·4
24	uninj. MCN ♀ *v.* uninj. MNC ♀	7·4	2·4
	uninj. MCN ♂ *v.* uninj. MNC ♂	2·9	1·8
	uninj. MCN ♀ *v.* CN ♀	6·6	2·6
	uninj. MCN ♂ *v.* CN ♂	4·0	2·3

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INDUCTION OF MULTIPLE PRIMARY TUMOURS IN FOWLS WITH 2-ACETAMIDOFLUORENE.

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THE carcinogenic property of 2-acetamidofluorene (N-acetyl-2-aminofluorene, acetylaminofluorene, 2-AAF) for fowls has been previously demonstrated by Bielschowsky and Green (1945), who reported the induction of renal tumours, and by Peacock and Peacock (1949, 1954). The latter recorded a squamous cell carcinoma of the crop mucosa which developed along the needle track following injection of 2-AAF in arachis oil into the lumen of that viscus ; and in their later paper described 3 cases of multiple primary tumours affecting various organs out of a total of 16 fowls which had received repeated injections of aqueous suspensions of 2-AAF in 25 mg. doses into the crop. In 4 other birds, single epithelial tumours developed.

Although spontaneous carcinomata are relatively common in older fowls (Olson and Bullis, 1942 ; Campbell, 1945), they have always been regarded as difficult to produce experimentally, compared, for example, with the ease with which they can be induced in the small rodents by various carcinogenic hydrocarbons. In particular, the carcinogenicity for rats of 2-AAF has been utilized experimentally by many workers since the first report by Wilson, De Eds and Cox (1941).

In contradistinction to experimentally produced mammalian carcinoma, those few which have been produced in fowls have not been transplantable, and indeed, no one has yet succeeded in propagating any epithelial tumour of the fowl by homologous transplantation, unless the limited success reported by Campbell (1949) with liver cell carcinoma in ducks is taken as an indication that the propagation of carcinomata in birds is not impossible. It follows, then, that experimental epithelial tumours of the fowl are of some interest, not only because of their rarity, but also because of the possibility of obtaining a carcinoma which might be propagated and which could then be subjected to similar extensive studies as the transplantable connective tissue tumours. The experiments now reported were begun in 1949, and, while on a small scale, have given very encouraging results, so that further work using different techniques is already going forward.

METHODS.

Eight Brown Leghorn pullets of an inbred strain and aged about 6 months were used. Five received daily doses of 25 mg. 2-AAF in 0·6 ml. sesame oil in gelatine capsules (Size No. 1, Parke Davis) orally for 3 months, Sundays excepted. The remaining 3 birds received sesame oil in capsules and served as controls. Treatment was commenced in February, 1949. The birds were kept in battery cages and fed the normal balanced ration used at the Centre.

Tissues taken at the end of the experiment were fixed in Susa, and prepared in the usual way for embedding in wax. Sections were cut at 5–7μ and stained with haematoxylin and eosin, or by Mallory's trichrome method. Where possible all fresh specimens were photographed immediately after post mortem examination.

Transplantation experiments consisted of intra-muscular, intra-peritoneal and intra-hepatic injections of finely-minced tumour tissues in normal saline. The tumours were either prepared for implantation direct from the post mortem material, or from fragments cultured on the chorioallantoic membrane of 10-day embryonated eggs, using the method of Beveridge and Burnett (1946).

RESULTS.

None of the birds showed any ill-effects as the result of the treatment, eating and drinking normally and coming into lay between 7 and 8 months of age. Normal moults were carried out, and the plumage pigmentation was unaffected. In May, 1953, one bird was found dead, having been laying intermittently for some time previously. Examination of the remainder showed another bird to be thin but in apparent good health, and the experiment was terminated by killing the remainder in the course of the next few days. Multiple tumours of the viscera were found in 3 birds out of the 5 treated with 2-AAF, and the other two showed cirrhosis of the liver and cystic kidneys. The controls were entirely normal. Table I summarizes the details.

TABLE I.—*Results of Treating Fowls with 2-AAF.*
All experiments commenced 1/2/49.

Fowl No.	Number of doses.	Total dosage 2-AAF (g.).	Result.
1	77	1·925	Died 7.v.53. Tumours in liver, intestine, oviduct, cysts and a tumour in one kidney.
2	77	1·925	Killed 10.v.53. Tumours in liver, ovary, oviduct and kidneys. Lesion in left lung.
3	77	1·925	Emaciated. Killed 10.v.53. Tumours in liver and ovary. Contracted granular kidneys.
4	77	1·925	Killed 10.v.53. Cirrhosis of liver. Cystic kidneys.
5	77	1·925	Killed 10.v.53. Cirrhosis of liver. Cystic kidneys.
6, 7, 8	77 (oil only)	—	Killed 12.v.53. No evident abnormality.

Post mortem findings and histopathology.

1. The liver was irregularly contracted, of a brownish colour and with prominent vessels visible beneath a thickened capsule. There were numerous bile-stained tumours, some discrete, others coalescent (Fig. 1). The gall bladder was

small and fibrosed, and contained a clear watery fluid. Small cream-coloured tumours were scattered on the serosa of the gizzard, duodenum and terminal part of the ileum. The left kidney was enlarged and cystic (Fig. 2), and one cyst contained an organizing blood clot. The right kidney was normal in size, but also cystic. The oviduct contained an isolated tumour which projected into the lumen of the magnum (Fig. 3). The ovary was that of an actively laying fowl, and all other viscera appeared normal.

Histological examination showed a fibrosis of the liver capsule with irregular bands of connective tissue entering the parenchyma, causing contracture and distortion. The capsular thickening was very pronounced in the region of the portal fissure and fossa for the gall bladder. There were numerous apparently benign hepatomata consisting of well differentiated but irregularly arranged cords of liver cells. Small zones of lymphocytes were fairly frequent in these tumours (Fig. 4).

The oviduct tumour was an adenocarcinoma arising from the mucosa of the magnum (Fig. 5, 6). Although this tumour was fairly well differentiated, with but little mitotic activity, it had spread by implantation to the serosa of the alimentary tract, as demonstrated by identical structure of those tumours.

Both kidneys showed multiple retention cysts, while the left organ contained a small encapsulated and trabeculated tumour composed of anaplastic cells bearing some resemblance to those of the oviduct tumour. The metastasis was apparently within a renal vein (Fig. 7). There was a pronounced concentric intimal fibrosis of the arterioles and the glomerular tufts were very cellular and hypertrophied (Fig. 8).

2. This bird ceased to lay early in 1953, and was killed 3 days after bird No. 1 died. It was found to have multiple liver tumours showing faint bile staining, and tumours in the ovary, oviduct and kidneys. A caseated abscess was present in the left lung.

Histologically, the liver tumours were typical hepatocellular carcinomata exhibiting patches of fatty change (Fig. 9). Metastases were present in both kidneys. The ovarian and oviductal tumours were adenocarcinomata of essentially similar structure, and it was difficult to ascertain which was primary. It seemed most likely, however, that the ovarian tumour (Fig. 10) represented a secondary deposit, since the structure resembled uterine glands more closely than it did a tumour derived from ovarian tissue.

Sections of lungs showed no tumours, but there was an old encapsulated and caseated abscess, and anthracosis was a prominent feature.

3. As this hen was becoming progressively more emaciated and had ceased to lay for several months, it was killed at the same time as bird No. 2. Post mortem examination showed multiple cream-coloured liver tumours, a small cystic ovary with a solitary tumour, and contracted granular kidneys. A large white nodule embedded in the left thyroid gland proved to be a hypertrophied parathyroid. The corresponding right gland was only slightly enlarged.

The liver tumours were hepatomata formed by relatively small undifferentiated cells arranged in irregular branching cords forming blood sinusoids (Fig. 11). The ovarian tumour was of an unusual nature and offered more difficulty in diagnosis. It consisted of a fairly abundant fibrous stroma, often forming the core of papillae which were invested with a single layer of cuboidal to columnar pale-staining epithelial cells with conspicuous vesicular nuclei. Loose accumula-

tions of similar cells were present in the pockets between papillae (Fig. 12). These frequently showed degenerative changes. Numerous cysts, lined with epithelial cells were present. A diagnosis of cystic papilliform adenocarcinoma of the ovary was made, but the origin of the epithelial cells was undetermined. Neither of these tumours metastasized. The kidneys showed a proliferative glomerulitis with interstitial infiltrations of granulocytes.

4, 5. These two birds were also killed at the same time. They had previously shown no symptoms, but No. 4 had ceased laying some months earlier, whereas No. 5 still laid sporadically. At examination, bird No. 4 was found to have a firm, somewhat smaller than normal liver and cystic kidneys. Histologically a mild cirrhosis was demonstrated, with regions of marked bile duct hyperplasia. The liver of bird No. 5 was also slightly cirrhotic, and the kidneys were cystic. All other organs appeared normal. No tumours were detected in either of these birds.

Controls.—The 3 controls, dosed with sesame oil only, were all in good condition and exhibited no evident abnormality upon post mortem examination.

Transmission Experiments.

Finely minced fragments of the liver tumour from fowl No. 1 were seeded on to the chorioallantois of 4 ten-day embryonated eggs. Tumours grew in all the eggs, and at 20 days they were opened, the tumours removed and finely divided with scissors and transferred to further eggs. These also grew, but did not reach such a large size at 20 days as in the first egg passage. One membrane tumour was taken for histology, and showed a well differentiated hepatoma, while the remainder were bulked, minced, suspended in normal saline, and injected into the breast muscles, peritoneal cavity, and livers of 6 six-week-old chicks. In all cases the tumour failed to take. Similar negative results were obtained in 6 young birds receiving fragments of the hepatoma obtained directly from the liver at autopsy.

Attempts were made to propagate both liver and ovarian tumours from fowl No. 3, with no greater success. As before, the direct homologous implants failed to grow, whilst tumour fragments grew satisfactorily on the C-A membrane for two passages. Subsequent efforts to implant the egg-grown tumours into six-week-old chickens were unsuccessful.

DISCUSSION.

Since the incidence of spontaneous tumours in the flock at the Poultry Research Centre is very low (1·6 per cent of all birds dying annually : Greenwood, Blyth and Carr, 1948) there is no doubt that dosing with 2-acetamidofluorene induced multiple primary epithelial tumours in 3 out of 5 birds treated. It is possible that tumours would have developed in the remaining 2 birds had they been allowed to live, for they both showed abnormalities of the liver and kidneys. When multiple tumours or concomitant neoplasia occurs in the human subject it is considered sufficiently unusual to merit reporting. A considerable number of papers are scattered throughout the pathological, surgical and obstetric journals, and it would be pointless to give details here, though it may be worthy of mention that the great majority of such reports do not discuss the implications of multiple primary tumours, except to speculate in a general way on "cancer-prone"

individuals. Spontaneous multiple primary tumours also occur in the lower animals, but are rarely reported. In the experimental field, however, numerous cases have been put on record.

Concomitant primary tumours in poultry are quite rare (Olson and Bullis, 1942). The present paper, together with those of Bielschowsky and Green (1945), and Peacock and Peacock (1949, 1954) demonstrates that, as in mammals, it is possible to induce multiple primary tumours in various organs of the fowl by means of a carcinogen. Some of these tumours were adenomatous, others frankly malignant, as proved by remote metastases and implantations. Indeed in fowl No. 2 there were present metastases of two distinct histological types. The propagation by transplants from certain of these tumours was unsuccessful, a not unexpected result, since past attempts at the transplantation of epithelial tumours of the fowl were not any more encouraging. However, two liver tumours and one ovarian tumour were grown on the chorioallantoic membrane of chick embryos for two passages, but failed to take in fowls when subsequently implanted. This suggests that future attempts at propagating epithelial tumours in the fowl should be made in very young chicks, even one day old.

In the present state of knowledge, speculations as to the ultimate cause of carcinogenesis is merely an exercise in unprovable hypotheses, and indeed, as Nicholson (1950) points out, we can know nothing of first principles. But it is tempting to look a little more closely into the implications of the development of multiple discrete tumours in an organ or tissue following the application of a carcinogen. Why, for example, should not the whole liver become diffusely cancerous when the animal is exposed to 2-acetamidofluorene, since all the hepatic cells are equally exposed over the period of administration? Instead, we see initial damage to the parenchyma, followed by regeneration, fibrous tissue proliferation, duct hyperplasia, and so on. This is succeeded by a gradual change in certain foci, where the cells pass insensibly from hyperplasia to neoplasia, forming the familiar nodules of a hepatoma, interspersed with apparently normal tissue (with the exception of a variable degree of cirrhosis). It seems reasonable to consider that these foci have arisen from cells differing fundamentally from all the remainder. Since they were presumably not originally different in either a pathological or embryonic sense (The Cohnheim-Ribbert doctrine being no longer tenable, as shown by Nicholson, 1950), the subsequent change may be due to the action of the carcinogen on a few cells in a particular and comparatively rare physiological state such as, for example, cell division. The mitotic rate is enormously increased following exposure of the liver to any substance which causes non-fatal damage, and a few of these dividing cells may undergo a particular mutation, bringing them to a precancerous phase. Such cells, exposed to the intermittent action of gonadotrophic hormones, may slowly differentiate through subsequent cell generations to true neoplastic cells no longer subject to the bonds of controlled physiological growth.

This is, of course, not an entirely new concept. It does, however, dispense with the cell-rest hypothesis, even now occasionally invoked to account for the focal nature of some tumours ; and it does embrace the various factors of extrinsic stimulus, chronic irritation, compensatory fibrosis, increased rate of cell division and hypertrophy, mutation, the pre-cancerous cell and intrinsic hormonal influence—all undoubtedly concerned in the initiation of the neoplastic state. Even the latent symbiotic " virus " as conceived by Rous (1936) could fit into the

scheme. Lastly, it helps to explain why such simple organic substances such as chloroform or carbon tetrachloride are liver carcinogens or such unlikely inorganic compounds as zinc chloride or sulphate are capable of causing teratomata to develop from the pluripotential germ cells in the testes of cockerels, and why successful induction is subject to seasonal influence (Michalowsky, 1928, 1929), or the simultaneous administration of gonadotrophic hormones subsequent to the spring months (Bagg, 1936).

SUMMARY.

Multiple primary epithelial tumours were obtained in 3 out of 5 fowls more than 4 years subsequent to a period of oral administration of 2-acetamidofluorene in sesame oil. These were hepatomata and carcinomata of the oviduct and ovary. Some of the tumours were propagated for a short time on the chorioallantoic membrane of fertile eggs, but none were successfully maintained in young chickens. The remaining two fowls had cystic kidneys and liver cirrhosis when killed at the termination of the experiment. Controls dosed with sesame oil alone remained normal. The significance of the focal origin of tumours after exposure of tissues to a carcinogen is discussed, and a synthesis of known factors in carcinogenesis is suggested to account for the sequence of events leading to the formation of a tumour.

All expenses in connection with this investigation were borne by the British Empire Cancer Campaign.

EXPLANATION OF PLATES.

FIG. 1.—Fowl No. 1. Liver, visceral surface, showing multiple hepatomata. The gall bladder has been removed. Natural size.

FIG. 2.—Fowl No. 1. Left kidney, showing several cysts and marked fibrotic changes. Natural size.

Fig. 3.—Fowl No. 1. Oviduct with isolated tumour projecting from the mucosa. Natural size.

FIG. 4.—Fowl No. 1. Histology of hepatoma. A mass of lymphocytes is at the bottom left. H. & E. × 230.

FIG. 5.—Fowl No. 1. Adenocarcinoma of oviduct. Trichrome. × 75.

FIG. 6.—Fowl No. 1. Detail of oviduct tumour. Trichrome. × 285.

FIG. 7.—Fowl No. 1. Metastasis of oviduct tumour within renal vein. Trichrome. × 285.

FIG. 8.—Fowl No. 1. Arterial fibrosis (sclerosis) and glomerular hypertrophy. Kidney. Trichrome. × 285.

FIG. 9.—Fowl No. 2. Hepatocellular carcinoma, with fatty change. H. & E. × 240.

FIG. 10.—Fowl No. 2. Adenocarcinoma in ovary, probably representing a secondary from the oviduct tumour, which was similar in structure. Trichrome. × 240.

FIG. 11.—Fowl No. 3. Hepatoma, showing smallness of cells, which form anastomosing cords and sinusoids. H. & E. × 240.

FIG. 12.—Fowl No. 3. Cystic papilliform adenocarcinoma of ovary. Trichrome. × 240.

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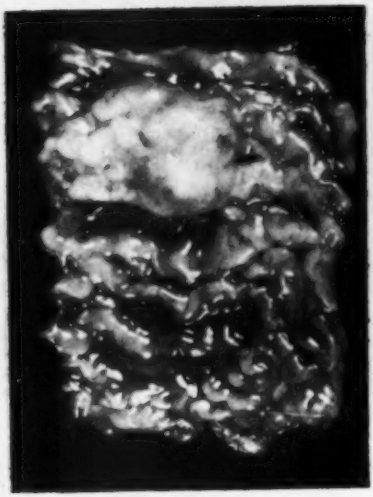

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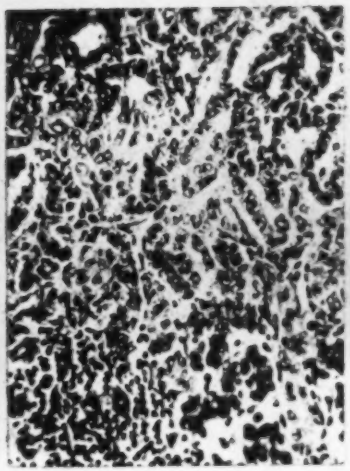

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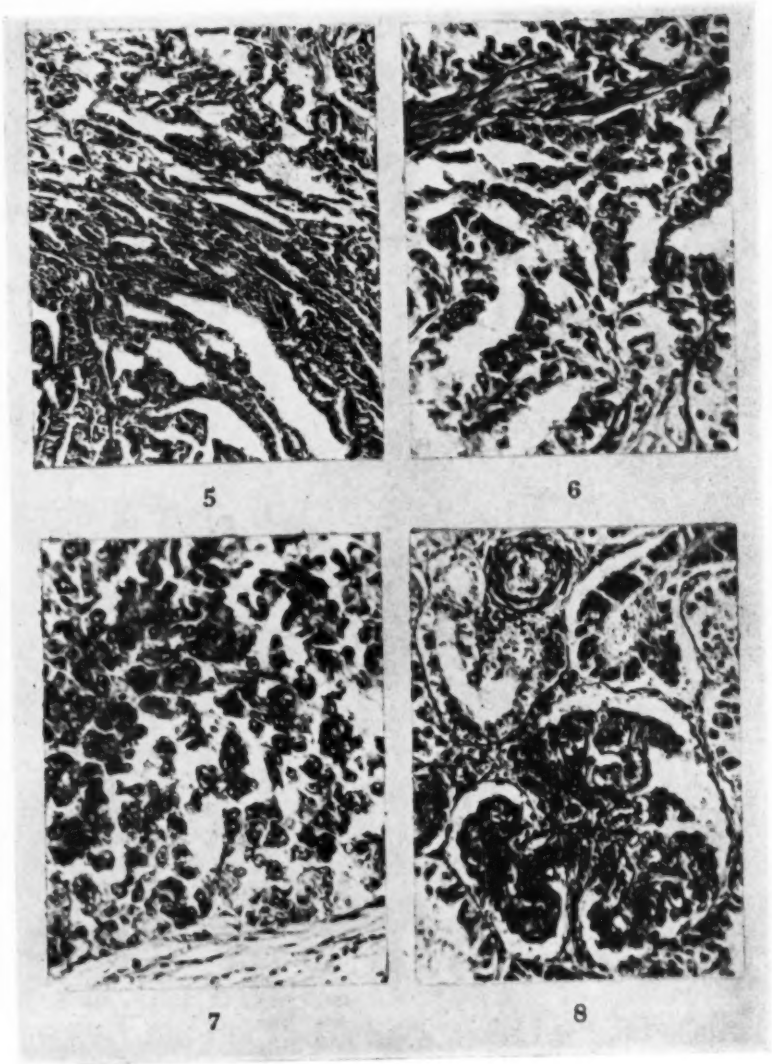

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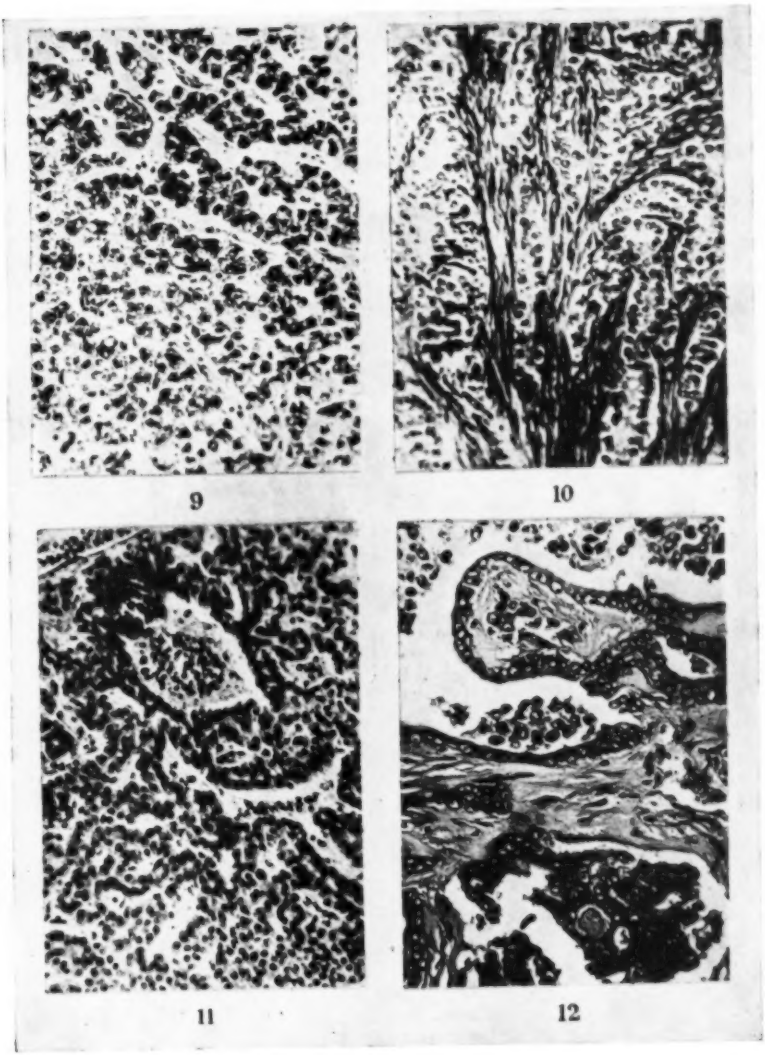

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BLADDER TUMOURS INDUCED IN RATS OF TWO STRAINS WITH 3 : 2′-DIMETHYL-4-AMINODIPHENYL.

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WE have recently shown that 4-aminodiphenyl is carcinogenic in rats and that certain of its derivatives containing a methyl substituent in the position ortho to the amino group are even more active in this respect (Walpole, Williams and Roberts, 1952). The most fully studied of the more active members of the series is 3 : 2′-dimethyl-4-aminodiphenyl (D.M.A.D.P.). This amine produces tumours in a variety of organs in rats from our colony (random-mated albinos of Wistar origin) but the yield of neoplastic lesions of the urinary bladder is very low.

Acetylamidofluorene (A.A.F.) resembles the carcinogens of the 4-aminodiphenyl series in its capacity to produce widespread neoplastic changes in the rat. Under normal dietary conditions, the yield of bladder lesions obtained with this substance appears to depend upon the strain of rat used. In the experiments in which its carcinogenic action was discovered, Wilson, de Eds and Cox (1941) worked with rats of the Slonaker strain. They observed a high incidence of hyperplastic and neoplastic changes in the bladder and renal pelvis, as well as in the liver, pancreas and lungs. Skoryna and Webster (1953) also have drawn attention to the susceptibility of Slonaker rats to the induction of bladder tumours with A.A.F. On the other hand bladder lesions rarely, if ever, result from its administration to Wistar or Sherman rats or to the piebald rats used by Bielschowsky, although it readily produces neoplastic changes in some other organs in animals of these strains (for summary and references see Kirby, 1947). Dunning, Curtis and Madsen (1947) studied the incidence of neoplasms in rats of five inbred strains given A.A.F. Bladder lesions were seen in 4 of the 10 treated Copenhagen rats and in 1 rat of the AxC strain ; none was found in those of the Marshall, August or Fischer strains.

Since D.M.A.D.P. produces so few neoplastic lesions of the bladder in our Wistar rats, it became of interest to determine its effect in a strain more prone to develop such lesions when given A.A.F. We selected Slonaker rats for this purpose. The results of the comparison have been reported briefly in a preliminary communication (Walpole, Williams and Roberts, 1954*a*). The bladder lesions obtained with D.M.A.D.P. are here described in greater detail and the results of the experiments more fully considered.

EXPERIMENTAL.

Most of our experiments conformed to the same general plan, the details of which were briefly as follows (see also Walpole, Williams and Roberts, 1952). The rats were about 3 months old at the start of experiment and were maintained

throughout upon a composite diet in pellet form with tap water freely available. They were weighed and dosed upon a weight basis five days a week throughout the period of dosing. D.M.A.D.P. was dissolved in arachis oil (B.P., sterilised by heating at 140° C. for 1 hour), and given by subcutaneous injection in the right and left pectoral regions respectively on alternate days. The dosing of individual rats was temporarily stopped if they lost weight and the length of the dosing period varied between groups according to the response observed. In most instances dosing was continued at levels near the maximum tolerated until the first tumours appeared.

In the one experiment in which the compound was administered in the food it was mixed thoroughly with powdered food pellets and the mixture given without further treatment.

Rats in control groups were given arachis oil alone in total doses greater than those used in corresponding groups for the administration of the compound.

The development of tumours was detected grossly by visual inspection (superficial and subcutaneous tumours) and by palpation (intestinal tumours, leukaemias and reticuloses). Diarrhoea often heralded the presence of intestinal tumours. Tumour-bearing animals which lost weight rapidly or became obviously ill were killed, and others were killed when they became moribund. Surviving rats in control groups were not sacrificed until the last in the corresponding treated groups had been killed.

At autopsy the bladders of all the rats were examined macroscopically. Most of them were distended with fixative solution (Zenker) and left in the fixative for some hours before being opened. In the earlier experiments bladder tissue was not always taken for section except when some abnormality was visible to the naked eye. Histological preparations were made from the distended bladders of 19 of the Slonaker rats given D.M.A.D.P., 1 to 4 different parts of each bladder being sampled. Only after gross lesions had been observed in this group were the bladders of all animals in other groups taken for section. As a result the incidence of microscopic abnormalities in groups other than this is not accurately known and the figures given below may represent an underestimate.

Parasitic worms, identified as *Trichosomoides crassicauda*, were found at autopsy in the bladders both of our Wistar rats and of those of the Slonaker strain. They were also seen in sectioned material. Their presence in the gross was not always recorded but the impression was gained (and supported by our findings in those bladders which were examined microscopically), that the incidence of the infestation was higher in the Slonaker rats than in the Wistars.

RESULTS.

The schedules of dosage, the mean survival times, and the incidence of parasitic worms and of epithelial lesions (most advanced lesions) in the bladders of both Wistar and Slonaker rats in the several groups given D.M.A.D.P., and in representative control groups, are shown in Table I.

Bladder lesions, when visible to the naked eye or under low-power magnification, ranged in appearance from rough thickenings to small and sometimes multiple cauliflower-like excrescences on the inner surface of the bladder wall. The outer surface invariably looked normal. Histologically, the earliest change consisted of slight thickening in areas of the bladder epithelium with an increase

TABLE I.—*Dosage Schedules, Survival Times and Incidence of Parasitic Worms and Epithelial Lesions in the Bladders of Control Rats and Rats Given D.M.A.D.P.*

Treatment.	Group.	Strain of rats.	Number and sex.	Mean duration of dosing (days).	Mean total dose (mg./100 g.).	Mean survival time (days).	Bladders examined microscopically.	Bladders with parasites.	Most advanced lesion. Slight epithelial thickening.	Most advanced lesion. Finger-like processes and plaques.	Most advanced lesion. Tumours.
Arachis oil alone (controls)	I	Wistar	11 ♂	292	—	470	10	1	—	—	—
	II	Wistar	11 ♀	292	—	499	6	1	—	—	—
	III	Slonaker	12 ♂	308	—	378	12	10	1	—	—
	IV	Slonaker	12 ♀	308	—	345	9	5	—	—	—
D.M.A.D.P. in arachis oil	V	Wistar	12 ♂	124	328	170	9	3	1+(1)	—	1
	VI	Wistar	12 ♂	141	276	183	6	2	—	—	—
	VII	Wistar	12 ♂	220	174	279	4	0	—	—	—
	VIII	Wistar	12 ♂	fed	430 approx.	275	4	2	—	—	1
	IX	Wistar	12 ♀	119	133	197	7	2	(1)	(2)	—
	X	Slonaker	12 ♂	249	230	270	9	6	—	—	8
	XI	Slonaker	12 ♀	233	235	247	10	1	3	2	4

Figures in parenthesis refer to bladders in which parasites were not seen.

in size of the individual cells, and in some instances hypereosinophilia of the cytoplasm. Changes of this nature were seen, in rats given the compound, in a total of 2 male and 1 female Wistars and 4 male and 9 female Slonakers. In 1 male Slonaker rat given arachis oil alone a small area of slight hyperplasia of the bladder epithelium was seen at the site of implantation of a parasitic worm. This was the only epithelial abnormality seen in control animals.

Rather more advanced lesions consisted either of individual finger-like processes projecting into the lumen of the bladder, or further thickening of the epithelium to form plaques (Fig. 1). In the former lesion, present in 1 male and 5 female Slonaker rats, the processes were covered with well-differentiated transitional epithelium and contained a connective tissue core. Plaques were seen in the bladders of 2 female Wistar and 2 male Slonaker rats, and in the latter short, well-circumscribed pegs of epithelial cells projected into the subjacent connective tissue.

Bladder tumours were found in 2 male Wistar rats and in 8 male and 4 female Slonakers. Papillomata, both sessile and pedunculated (Fig. 2) were seen, and one of each type account for the only two tumours found in the Wistar rats. In four sessile growths there was squamous metaplasia in parts of the epithelium (Fig. 3), and in two pedunculated tumours early malignant change had taken place. Where this had occurred there was an area of cells showing poor differentiation and irregular arrangements and groups of cells had infiltrated locally into the stroma of the tumour (Fig. 4 and 5). In addition, sessile infiltrative growths were present in the bladders of 2 male and 2 female rats (Slonaker). These

EXPLANATION OF PLATES.

FIG. 1.—Epithelial plaque in the bladder of a Slonaker rat. × 75.

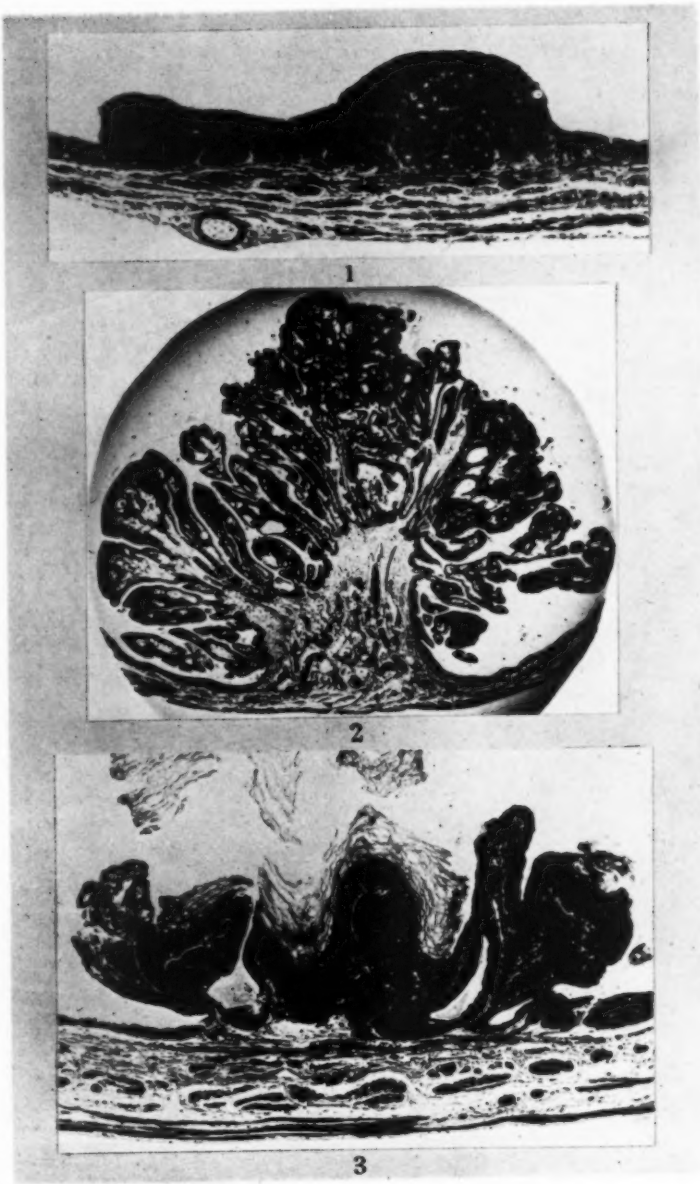
FIG. 2.—Papilloma in the bladder of a Wistar rat. × 13.

FIG. 3.—Sessile papilloma in the bladder of a Slonaker rat. Note squamous metaplasia. × 75.

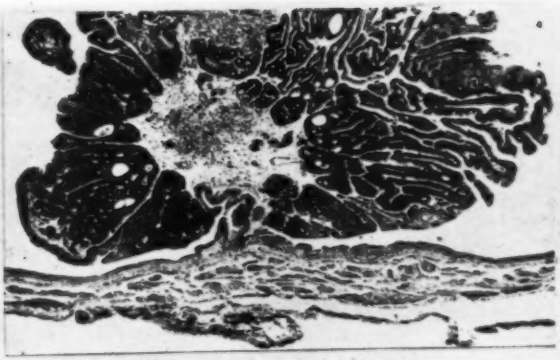
FIG. 4.—Papilloma in the bladder of a Slonaker rat. Note area of early malignant change. × 20.

FIG. 5.—Detail of papilloma shown in Fig. 4. Note invasion of stroma by transitional cells. × 72.

FIG. 6.—Infiltrating tumour in the bladder of a Slonaker rat. × 72.



Walpole, Williams and Roberts.



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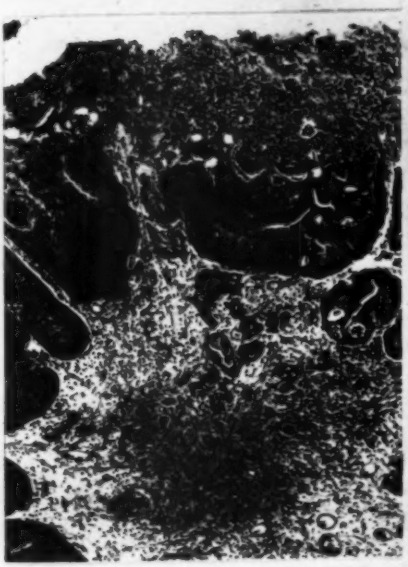

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consisted of masses of poorly differentiated transitional cells on the surface of which, in three instances, short, blunt, papillary processes were seen. On the deep surface, columns of epithelial cells had infiltrated the subjacent connective tissue (Fig. 6). In no instance was the muscle of the bladder wall involved.

Neoplasms occurred in a variety of other organs in rats given D.M.A.D.P. The histological appearance of those obtained in our Wistar strain has already been described (Walpole, Williams and Roberts, 1952) and those occurring in the Slonaker rats were essentially similar. Of the more common of these other neoplasms, only those affecting the intestinal tract had a marked influence upon survival time and will be considered further in the present context. The survival time of individual animals in Groups VI (male) and IX (female) of our Wistar rats, and in the two Groups X (male) and XI (female) of Slonakers are represented in the diagram (Fig. 7), together with the incidence of hyperplastic and neoplastic changes in the intestines and bladders of these animals.

DISCUSSION.

In the experiments recorded above we obtained a much higher yield of bladder lesions—ranging from mild epithelial proliferation to tumours infiltrating subjacent connective tissue—in Slonaker rats given D.M.A.D.P. than in rats of our own Wistar strain. In this respect our results resemble those obtained by other workers with A.A.F. Skoryna and Webster (1953) have observed proliferative and chronic degenerative changes in the bladder wall of Slonaker rats of the Stanford strain, both in untreated animals and in those receiving A.A.F. in the diet, and found bladder stones in several of their rats. They entertain the possibility that these lesions may have a localising effect on the action of this carcinogen, causing tumour development in a previously injured tissue. In none of the bladders, from rats of either strain, examined in the course of our experiments were any pathological changes seen other than those described above, and no bladder stones were found. In untreated animals the only difference noted was the rather higher incidence of parasitic infestation in the bladders of the Slonaker rats. Fig. 7 reveals that the rate of appearance of bladder lesions was, in fact, not very different in rats of the two strains given D.M.A.D.P. However, all the male and most of the female Wistar rats were dead before the first frank tumour appeared in the corresponding group of Slonakers. The most significant feature of our results, as is clear from this diagram, is the comparatively high incidence of intestinal tumours occurring early in the Wistar rats. In the experiments in question, every one of the 12 male and 6 of the female Wistars had developed tumours of the intestine by 270 days, at which time a similar tumour had been detected in only 1 (male) rat of the 12 male and 12 female Slonakers. Even at 375 days, when the last of the 24 Slonaker rats was killed, intestinal tumours had been found in only 7, despite the fact that the total dose of carcinogen received by the males of this strain was about the same as that given to the Wistar males, while the females had almost twice that given to the females of the Wistar strain.

In the experiments of Dunning, Curtis and Madsen (1947), in which A.A.F. was given to rats of five different strains, the results were similar. Bladder tumours were observed in the highest frequency in animals of the strain (Copenhagen) which, as a group, survived the longest. Rats of other strains died

earlier, mostly with hepatic lesions, before bladder tumours appeared. It would be interesting to know the survival times of the Slonaker rats in Skoryna and Webster's (1953) experiments, and the dose of A.A.F. given, in comparison with the results obtained in other strains. Later experiments by Dunning, Curtis and Maun (1950) indicate that factors other than survival time are involved in the variation in response to A.A.F. When this carcinogen was given to Fischer rats on diets containing casein (26 to 45 per cent) as the protein source, no bladder

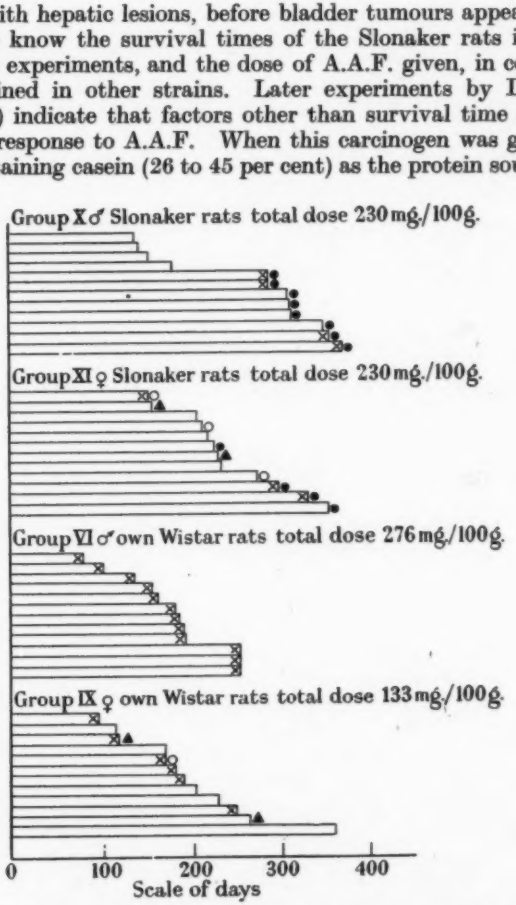
Group X ♂ Slonaker rats total dose 230 mg./100 g.
Group XI ♀ Slonaker rats total dose 230 mg./100 g.
Group VI ♂ own Wistar rats total dose 276 mg./100 g.
Group IX ♀ own Wistar rats total dose 133 mg./100 g.
0
100
200
300
400
Scale of days

FIG. 7.—Incidence of intestinal tumours and bladder lesions in relation to survival time in individual rats of two strains given D.M.A.D.P. ×] Intestinal tumours.]○ Slight epithelial thickening in bladder.]▲ Finger-like processes or plaques in bladder.]● Bladder tumours.

tumours were obtained. When it was given in a low casein (hydrolysate) diet containing in addition 1·4 per cent of DL-tryptophane, bladder tumours appeared and the survival time was indeed prolonged. With 4·3 per cent of tryptophane in the diet, however, the mean survival time was not greatly extended (320 days as against 283 days on the low casein diet and 303 days on the high casein diet) but carcinoma of the bladder developed in 11 of the 12 treated animals.

In the intestinal tract of the control rats of our Wistar strain, we were unable to find any chronic pathological change which might localise the action of a carcinogen. The intestines appeared normal and indistinguishable from those

of untreated Slonaker rats. Some other explanation is needed for the comparatively early appearance of tumours in this site in our Wistar rats given D.M.A.D.P. It may be that the intestinal tract of these rats has some inherent susceptibility to the action of the carcinogen, the nature of which is as yet quite unknown. There is, however, an alternative explanation which merits further investigation. We have already suggested (Walpole, Williams and Roberts, 1952) that 4-aminodiphenyl and its methylated homologues have no carcinogenic action *per se* but are metabolised in the body to effective carcinogens. We have suggested further that the occurrence of intestinal tumours is due to the appearance of these carcinogenic metabolites in the bile. It may well be that rats of the Slonaker strain excrete relatively less of these metabolites by way of the bile. We suspect that the metabolites in question are ortho-hydroxy amines (cf. Clayson, 1953).

The results obtained by various workers with A.A.F. could perhaps be explained along the same lines, and the effect of dietary tryptophane may be to alter the metabolic pathways of this substance. It is interesting to note that Dunning, Curtis and Segaloff (1947) propose a metabolic explanation for the difference in yield of various tumours in rats of different strains given diethylstilboestrol. They cite evidence that the metabolism of oestrogens varies from strain to strain.

It remains to consider the cause of the bladder lesions in Slonaker rats given D.M.A.D.P. In reporting the production of bladder tumours in rats by the introduction of pellets of paraffin wax into the bladder lumen, Bonser, Clayson, Jull and Pyrah (1953) suggest that chronic, non-specific irritation may suffice to induce malignancy in this species. Bladder calculi have been observed in rats in which the administration of A.A.F. has resulted in tumours of the bladder; calculi would always seem to be present where such tumours result from giving oestrogens (Dunning, Curtis and Segaloff, 1947, 1953). Fitzhugh and Nelson (1946) report a close correlation between their occurrence and tumours in the bladder of rats given diethyleneglycol. We found no calculi in the bladders of the rats examined in the course of our experiments. *Trichosomoides crassicauda* was seen but does not itself appear to cause tumours. The infestation was heavy in the bladders of our Slonaker controls in which no tumours were found, and, similarly, Spitz, Maguigan and Dobriner (1950) found no lesions due to this parasite in heavily infested rats of the Sherman strain. Where we found neoplastic bladder lesions, the parasite was almost always seen, and may have played some part in their genesis. It should be remembered, however, that D.M.A.D.P. produces a variety of other tumours in rats. Moreover it is closely related to 4-aminodiphenyl itself which in addition to yielding tumours of different tissues in the rat is also very effective in the production of bladder cancer in the dog (Walpole, Williams and Roberts, 1954*b*). Taking these facts into account we regard our rat bladder lesions as essentially due to the action of the compound. We believe, however, that the occurrence of bladder tumours only in rats treated with a compound is not a reliable indication of carcinogenic activity if it is associated with the appearance of concretions in the bladder.

SUMMARY.

A high incidence of bladder tumours has been obtained in Slonaker rats as compared with rats of Wistar descent when both were given 3 : 2′-dimethyl-4-

aminodiphenyl by subcutaneous injection. Further analysis of the results reveals that while the Wistar rats died early from intestinal tumours such neoplasms were rare in the Slonaker rats. The latter survived much longer and in them, at a later date, bladder tumours developed in high yield. The rate of appearance of such lesions was not, in fact, very different in rats of the two strains.

No pre-existing lesions were found in the intestinal tract of the Wistar rats such as might localise the action of a circulating carcinogen, and an alternative explanation of the strain difference is suggested.

The factors involved in the induction of bladder tumours in rats are discussed.

We wish to thank Mrs. B. Hubbert and Miss P. Nutty for their help with these experiments.

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FURTHER STUDIES ON INCOMPLETE CARCINOGENESIS: TRIETHYLENE MELAMINE (T.E.M.), 1,2-BENZANTHRACENE AND β-PROPIOLACTONE, AS INITIATORS OF SKIN TUMOUR FORMATION IN THE MOUSE.

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In previous papers (Salaman and Roe, 1953; Roe and Salaman, 1954) it was shown that the application of urethane (ethyl carbamate) to the dorsal skin of mice followed by repeated applications of croton oil gave rise to many epidermal tumours, some of which were malignant,* and that the number of tumours which appeared was roughly proportional to the total dose of urethane applied. Urethane alone, applied repeatedly in large doses, produced no tumours, nor any recognisable histological change in the skin. Repeated application of croton oil alone produced very few tumours. Repeated application of urethane following a single "initiating" dose of a carcinogenic hydrocarbon failed to produce any skin tumours.

The object of these experiments was to test the hypothesis that there exist "incomplete carcinogens" which act as "initiators" but not as "promoters" of carcinogenesis. The meaning of these terms was defined. It was concluded that urethane was such an incomplete carcinogen for mouse skin.

2-Acetylaminofluorene, p-dimethylaminoazobenzene (butter yellow), and NN-di-(2-chloroethyl)-β-naphthylamine (R48), were tested by a similar technique, but none of these showed any initiating power. Methyl bis (β-chloroethyl) amine hydrochloride (nitrogen mustard) gave an equivocal result. This substance has been re-examined, and the result is included among those reported here.

In an attempt to find other initiators of carcinogenesis a further 28 substances have been screened for this property by methods essentially similar to those previously used. The majority were selected from the very large number of substances which possess one or more biological activities in common with urethane, or which are related to it chemically.

Selection of substances.

A list of substances screened is given in Table I. It was found convenient to divide them into classes, but it is not implied that the class-names completely describe the biological activity of each substance (e.g. nitrogen mustard is classified as an antileukaemic agent, but could equally well be classed among the substances known to exert a specific effect on cells undergoing mitosis).

* Among 45 mice which survived until the end of treatment with various doses of urethane in conjunction with croton oil there have been 5 carcinomata and 1 sarcoma. These have appeared 6–13 months from the beginning of treatment.

The first four substances which we have examined are classed as *antileukaemic agents*. These are of particular interest because of the frequent association between inhibition of tumour growth and carcinogenesis (Haddow, 1935, 1938 ; Haddow and Robinson, 1937, 1939 ; Haddow, Scott and Scott, 1937 ; Badger *et al.*, 1942).

The antileukaemicactivity of urethane itself is well authenticated both in human and animal leukaemia (Paterson *et al.*, 1946 ; Engstrom, Kirschbaum and Mixer, 1947 ; Berman and Axelrod, 1948).

1,4-Dimethanesulphonoxybutane (Myleran) was first synthesized and shown to be an antileukaemic agent by Haddow and Timmis (1951, 1953). It is now widely used in the treatment of human leukaemia, and is especially valuable in the chronic myeloid variety (Galton, 1953 ; Petrakis *et al.*, 1954 ; Boyland, 1954). Koller (1953 ; 1954, personal communication) produced sarcomata in rats by the subcutaneous injection of Myleran, and commented on the abnormal mitotic figures in these tumours.

Triethylene melamine (T.E.M.) was synthesized simultaneously at three centres (Haddow, 1950 ; Rose, Hendry and Walpole, 1950 ; and Burchenal *et al.*, 1950), and has found a place in the treatment of lymphatic leukaemia (Paterson, Kunkler and Walpole, 1953 ; Bayrd *et al.*, 1952). Rose *et al.* (1950) commented on the cytotoxic effects of T.E.M., and compared them with those due to nitrogen mustard, as described by Gaensler *et al.* (1948). There is evidence that T.E.M. possesses carcinogenic activity under certain conditions. Walpole *et al.* (1954) report the early development of sarcomata in rats following twice-weekly subcutaneous injection of T.E.M. in arachis oil (total dosage : 0·7 to 1·1 mg./100 g.). Shimkin (1954) reports an increased incidence of pulmonary adenomata in mice treated with T.E.M. Hendry *et al.* (1951) reported an increased incidence of lung tumours in mice injected intraperitoneally with T.E.M., but failed to induce sarcomata in mice by repeated subcutaneous injection of the substance (0.025 mg. weekly for over a year). Lewis and Crossley (1950) found that T.E.M. possessed tumour-inhibitory activity against sarcomatous implants, and also reported a decreased incidence of spontaneous lung tumours in the treated mice. Graffi, in a review (1953) of his work and that of his colleagues, described briefly an experiment in which mice were painted with T.E.M. and croton oil. No tumours resulted from this treatment.

Nitrogen mustard has been known as an antileukaemic agent since the end of the last war, when a leucopenia was observed in men accidentally exposed to it (Goodman *et al.*, 1946). Its present role in the therapy of leukaemia is reviewed by Gellhorn (1953), Wilkinson, Haddow and Nabarro (1953), and Boyland (1954). The carcinogenicity of nitrogen mustard has been investigated by several workers. Boyland and Horning (1949) reported that 10 out of 14 mice injected subcutaneously with nitrogen mustard (1 mg./kg. weekly for 36 weeks or more) developed tumours (8 lung adenomata, 2 lymphosarcomata, 1 uterine fibromyoma, and 1 spindle-cell sarcoma at the injection site). Griffin, Brandt and Tatum (1951) induced similar tumours after injecting nitrogen mustard subcutaneously, intraperitoneally, or intravenously. Heston, Lorenz and Deringer (1953), and Shimkin (1954), induced pulmonary adenomata in mice with nitrogen mustard. Narpozzi (1953) tested nitrogen mustard for initiating activity on mouse skin, using croton oil as the promoting agent. To 60 mice he gave 15 applications of 0·05 per cent nitrogen mustard on alternate days, and after an interval of 10 days he began applications of croton oil (5 per cent in liquid paraffin) ; the latter were given on

alternate days for over a year. During this period 6 mice developed up to 5 warts each, but no malignant tumours were observed. In our experience this number of tumours may well have been due to the croton oil itself (see p. 185). The author did not include a group of mice painted with croton oil only.

Petering (1952), Gellhorn (1953), and Boyland (1954), review the status of aminopterin in the treatment of malignant disease, including leukaemia. Aminopterin has been shown to exert a specific effect on mitosis (Hughes, 1950). There are no reports ascribing any carcinogenic action to aminopterin, or to related folic acid analogues.

The second class of substances has been designated as *exerting a specific effect on cells undergoing mitosis.*

An effect of urethane itself on the process of mitosis was reported by Ludford (1936). He observed abnormal metaphases following treatment of tissue cultures with 2 per cent urethane. Lasnitzki (1949) studied the effect of 0·4 per cent urethane on growth and mitosis in cultures of normal and malignant tissues from the mouse. The growth and mitotic rates of normal tissues were reduced to approximately half the control levels. Cultures of C57 sarcoma and adenocarcinoma 63, however, showed enhancement of growth, and mitotic rates of 2 to 4 times the control values. An increased number of abnormal cell divisions were seen in the adenocarcinoma cultures. Dustin, P. (1947*b*), studied the effect of urethane on the epithelium of the intestinal crypts, and observed an effect similar to that caused by X-irradiation. He therefore placed urethane in the class of "*radiomimetic agents*" (Dustin, A. P., 1929). More recently Loveless and Revell (1949) have pointed out that there is little evidence that urethane gives rise to the irregular chromosome-breakage which characteristically appears 18–24 hours after exposure to X-irradiation.

The arrest of mitosis in metaphase by colchicine is well known (see Ludford (1953) for review).

Podophyllin exerts a similar action (King, 1948 ; King and Sullivan, 1946).

Cornman (1947) claimed that coumarin produced metaphase-arrest in the roots of *Allium cepa* and *Lilium longiflorum*. Ostergren (1948) found that coumarin produced chromosome bridges and breaks in *Allium cepa*. Since this substance is commonly used in some countries as a flavouring agent in tobacco (Vasic, 1953), we thought it worth while including in the present series. It is a lactone (see p. 182).

The evidence that hydroquinone belongs to this class is dependent on the findings of Zylberszac (1939), and Dustin P. (1947*a*), in the epithelium of the intestinal crypts of the mouse. The latter concluded that hydroquinone exerted a radiomimetic action. Loveless and Revell (1949), on the other hand, doubt whether hydroquinone should be regarded as exerting a specific effect on mitosis.

Sodium cacodylate was one of the organic arsenical compounds studied by Dustin and his collaborators (Piton, 1929 ; Dustin and Piton, 1929 ; Dustin and Grégoire, 1933), and was observed to give rise to metaphase-arrest indistinguishable from that due to colchicine.

None of the substances included in this category can be regarded as definitely carcinogenic for mouse skin. Harde (1939) applied colchicine to the skin of 26 mice weekly. Three mice eventually developed tumours of the skin, a further 2 mice developed tumours of other organs. Hamperl (1946) produced no tumours in mice after painting with colchicine. Berenblum (1951) tested podophyllotoxin on mouse skin, and concluded that it possessed slight anticarcinogenic, but no

carcinogenic or co-carcinogenic activity; Gwynn and Salaman (1953) found no evidence of co-carcinogenic activity.

A third class of substances with *narcotic* properties were tested for power to initiate carcinogenesis. They resemble urethane not only in their action on nervous tissue, but also in retarding or suppressing mitosis (Ludford, 1953, for review); and the possibility was considered that there might be some connection between these properties and that of initiation of carcinogenesis. Urethane was one of the narcotics used by Quastel and his associates in their study of the chemical mechanism of hypnotic action (Quastel and Wheatley, 1933, 1934; Michaelis and Quastel, 1941). They showed that these substances all have an inhibiting effect on aerobic glycolysis by brain tissue *in vitro*. The effect is reversible, i.e. when the drug is removed by washing the greater part of the glycolytic activity reappears. Indole also has a powerful inhibitory effect on aerobic glycolysis of brain *in vitro*; but in this case activity is not restored by washing the tissue (Quastel and Wheatley, 1934).

A group of narcotics was chosen for test which included members of a wide variety of chemical types (see Table I, Class III). Indole was also tested (Class VI), for the reason given above.

Following the discovery by Nettleship and Henshaw (1943) of the lung tumour-inducing property of urethane, Larsen, Rhoads and Weed (1946) examined a series of hypnotics for the same property (including chloral hydrate, paraldehyde, and phenobarbitone). None of the substances tested increased the incidence of pulmonary tumours in mice.

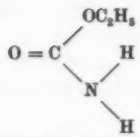
The fourth class of substances has only two members, both of which possess a *pharmacological action in some respect similar to urethane*, though they cannot be included in the preceding classes.

o-Phenanthroline inhibits the photochemical decomposition of water by chloroplasts *in vitro*, and photosynthesis *in vivo* (Warburg and Luttgens, 1944; Arnon and Whatley, 1949). A similar inhibitory action has been ascribed to the urethanes as a group (see Gaffron and Fager, 1951, for review).

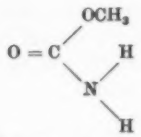
Physostigmine was included because its activity as a choline-esterase inhibitor has been attributed to the urethane group in its molecule, although urethane itself is inactive in this respect. (Stedman and Stedman, 1931, 1932; Eadie, 1942).

The substances in the fifth class were chosen because they could be regarded as *urethane derivatives* or in some way *structurally related to urethane*. Their structural formulae are shown in Fig. 1.

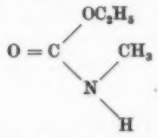
Other workers have examined urethane analogues from different standpoints. Lefèvre (1939), and Simonet and Guinochet (1939), described a colchicine-like effect in plant cells due to treatment with ethyl N-phenylcarbamate (phenylurethane). Dustin (1947*b*) found that ethyl N-phenylcarbamate was more toxic than urethane, and caused similar changes in mouse intestine, whereas methylcarbamate was of low toxicity and caused no narcosis or mitotic disturbance. Larsen (1947*a*, 1947*b*, 1948), studying the narcotic and lung tumour-inducing activities of urethane analogues, concluded that methylcarbamate was inactive in both these respects, and that increasing N-alkylation led to decreasing lung tumour-inducing activity. He considered that N-alkylated urethanes probably acted only after dealkylation to urethane in the body. Skipper *et al.* (1948) made an exhaustive study of urethane derivatives, including methylcarbamate, ethyl-



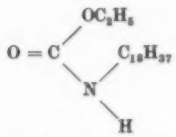
Ethyl carbamate (urethane).



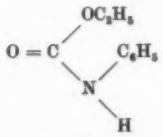
Methyl carbamate (Group 30).



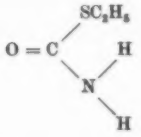
Ethyl N-methyl carbamate (Group 31).



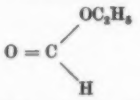
Ethyl N-octadecyl carbamate (Group 32).



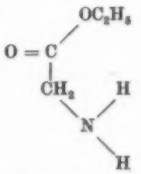
Ethyl N-phenyl carbamate (Group 33).



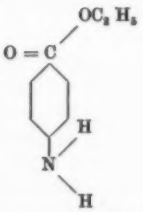
Ethyl thiolcarbamate (Group 34).



Ethyl formate (Group 30).



Glycine ethyl ester (Group 35).



Benzocaine (p-amino ethyl benzoate) (Group 29).

FIG. 1.—Structural formulae of urethane, urethane derivatives, and related compounds. (Group numbers refer to Table I, Class V.)

thiolcarbamate, ethyl N-methylcarbamate, and ethyl N-phenylcarbamate. The toxicity of these four, except methylcarbamate, if expressed on a molar/body weight basis, was greater than that of urethane. Methylcarbamate alone showed no anaesthetic properties. Ethyl N-methyl carbamate and ethyl N-phenylcarbamate both caused leucopenia when administered in non-lethal doses, but neither produced such a striking reversal of the differential leucocyte count as urethane. Rose *et al.* (1950) examined some thirty simple homologues of urethane for growth inhibitory activity against the Walker carcinoma 256 in rats. Of these only *bis*- and *tris*-carbethoxyamine even approached urethane in activity.

Benzocaine, apart from its structural relation to urethane (Fig. 1), is of interest in that it has a slight local anaesthetic action. Moreover it is an ester of p-aminobenzoic acid, and thus a potential inhibitor of the action of the sulphonamides. A similar action has been ascribed to urethane (Johnson, Eyring, and Kearns, 1943).

The subcutaneous injection of very large doses of ethyl N-octadecylcarbamate in rats has been found to produce sarcomata (Walpole, 1953, personal communication). Because of this result we thought it worth while testing as an initiator.

The sixth class consists of four *miscellaneous* substances, 1,2-benzanthracene, cantharidin, indole, and β-propiolactone.

1,2-Benzanthracene was selected mainly in order to repeat the work of Graffi *et al.* (1953). In their experiments 75 mice received alternate applications (each of 3 drops) of 0·5 per cent 1,2-benzanthracene in acetone and 5 per cent croton oil in liquid paraffin, at 3 to 4 day intervals. This treatment was continued for a year, by which time there were 18 benign skin tumours in the 9 surviving mice. At the same time 13 survivors of 160 mice, which had been treated with croton oil only, showed only one tumour. Previously Berenblum (1941) recorded a negative result from a similar experiment, in which two groups of mice were painted weekly, one with a saturated solution of 1,2-benzanthracene in acetone, and the other with a similar solution containing also 0·025 per cent croton oil resin. No tumours were present in either group after 20 weeks of continuous treatment.

Steiner and Falk (1951) reported the production of 8 sarcomata in 44 mice injected subcutaneously with 5 mg. of 1,2-benzanthracene, and in the same paper reviewed the findings of other workers on the carcinogenicity of this substance. Isolated tumours of mouse epidermis, the tissue in which we are primarily interested, have been seen following skin application of 1,2-benzanthracene (Kennaway, 1930; Cook, 1933; Barry *et al.*, 1935; and Hill *et al.*, 1951).

As pointed out by Steiner and Falk (1951) 1,2-benzanthracene is of interest in the study of the relationship between carcinogenic potency and molecular structure, because of its borderline position both in the scale of carcinogenicity and that of physico-chemical properties (e.g. electron density at the "K" region, photodynamic effects, and ultraviolet absorption spectrum) which have been correlated with carcinogenicity (Mottram and Doniach, 1938; Jones, 1940; Koffler and Markert, 1951; Badger, 1954 (review)).

The inclusion of indole has already been discussed in connection with substances of Class III (*vide supra.*)

β-propiolactone $\left\{\begin{matrix} CH_2—CH_2 \\ | \quad\quad | \\ O——CO \end{matrix}\right\}$ was found to possess mutagenic activity in *Neurospora* (Smith and Srb, 1951). Walpole *et al.* (1954) found it to be carcinogenic for the subcutaneous tissue of the rat. The latter authors commented on the resemblance in chemical properties between this substance and ethyleneimine. In view of these reports, and the simple nature of the compound, we thought it worth including in the present series of tests.

Cantharidin, a powerful skin irritant, reduced the carcinogenic effect of tar when applied alternately with the latter to mouse skin; in this respect it resembled mustard gas (Berenblum, 1935). Orr (1938) tested cantharidin for carcinogenic action on mouse skin and obtained no tumours. Gwynn and Salaman (1953)

tested it for co-carcinogenic activity, with a negative result. The possibility that this intensely toxic substance might be an initiator of carcinogenesis had thus not been excluded. In view of the properties of β-propiolactone it is worth noting that cantharidin is also a lactone.

Choice of dose and mode of application.

It was not found possible to follow a uniform scheme of dosage throughout.

In the case of the chemical analogues of urethane the object was to compare their initiating power with that of urethane itself; they were therefore used in quantities molecularly equivalent to, or greater than, a dose of urethane the effect of which was known. Fortunately this could be done without interference by toxic effects. An exception was ethyl N-octadecyl carbamate, of which an equimolecular dose would have been difficult to apply, and which was included because of the results of Walpole (1953, personal communication) already referred to.

In the case of nitrogen mustard, which had given a result of doubtful significance in a previous experiment (Salaman and Roe, 1953), a dose was used which produced most tumours in the former test.

In the other groups, whenever possible, the largest doses which just failed to produce toxic symptoms, or other signs of general effect, e.g. narcosis, were used. In some cases, however, the limit was set by the maximum attainable concentration in a suitable solvent, in others by the fact that strong solutions left heavy deposits of solid substance as the solvent evaporated, which were rubbed off without penetrating the skin.

The mode of application also varied to some extent. In the early stages of the work it was thought that repeated doses of the initiator given alternately with croton oil would probably produce more tumours than one, or perhaps two, applications of the initiator followed, after an interval, by weekly applications of croton oil. Experience has shown that initiation is either demonstrable by both methods (e.g. urethane, T.E.M.) or not demonstrable by either (e.g. nitrogen mustard, chloral hydrate). The methods are therefore to be regarded as qualitatively equivalent.

Details of dosage and methods of application are given in the following section, and in Table I.

MATERIALS AND METHODS.

Mice.

Stock albino male mice of the " S " strain (Salaman and Gwynn, 1951; Salaman and Roe, 1953), fed on cubes prepared according to the Rowett Institute formula (Thomson, 1930*a*, 1930*b*) plus fresh greenstuff twice a week, and water *ad libitum*, were used throughout. They were vaccinated on the tail with sheep lymph (kindly supplied by the Lister Institute of Preventive Medicine) as a precaution against ectromelia. Only positive reactors were used. At the beginning of experiments they were 7–9 weeks old.

Chemical substances and solvents.

Test substances.—Benzocaine (ethyl ester of p-amino benzoic acid), cantharidin, carbromal (1-bromo-ethyl-butyryl-urea), chlorbutol (1 : 1 : 1-trichloro-2-methyl-2-propanol), coumarin, ethyl carbamate (urethane), ethyl formate (ethone), ethyl

N-phenylcarbamate (phenylurethane), glycine ethyl ester hydrochloride, hydroquinone, indole, methyl carbamate, methyl sulphonal, ortho-phenanthroline (AR grade), physostigmine (eserine), and sodium cacodylate, were obtained from British Drug Houses, Ltd.

1,2-Benzanthracene, colchicine, and β-propiolactone were obtained from Messrs. L. Light and Co., Ltd. ; chloral hydrate from T. H. Smith and Son, Ltd. (Edinburgh) ; nitrogen mustard (HN2, methyl bis (β-chloroethyl) amine-hydrochloride) from Messrs. Merck and Co. ; and phenobarbitone (luminal) from Messrs. Huffer and Smith, Ltd. (Croydon, Surrey).

Paraldehyde (2,4,6-trimethyl-1,3,5-trioxane), and podophyllin resin (for preparation of podophyllin extract, see below) were obtained from Messrs. Allen and Hanburys, Ltd.

Dr. Walpole of Pharmaceutical Division, Imperial Chemical Industries, Ltd., kindly supplied ethyl N-methylcarbamate, ethyl N-octadecylcarbamate, ethyl N-thiolcarbamate (thiourethane), and triethylene melamine (T.E.M., tris ethyleneimino-s-triazine).

Mr. G. Timmis of the Chester Beatty Institute, London, kindly supplied us with Myleran (GT 2041, 1 : 4-dimethane-sulphonoxy butane).

Aminopterin (4-amino-pteroylglutamic acid) was kindly supplied to us by Cyanamid Products, Ltd., Lederle Laboratories Division.

Croton oil.—Expressed oil of the same batch used in previous experiments in this laboratory (e.g. Salaman and Gwynn, 1951 ; Salaman and Roe, 1953) was used. It was obtained from Messrs. Boots Pure Drug Co., retailing the product of Messrs. Stafford Allen and Sons, Ltd., 20 Wharf Rd., N.1.

Solvents.—Acetone (AR grade), and ether (Aether Puriss. grade) were obtained from British Drug Houses, Ltd., carbowax 300 (polyethylene glycol of average molecular weight 300) from General Metallurgical and Chemical Co., Ltd., and methyl alcohol from James Burrough, Ltd.

Preparation of solutions.

All solutions were prepared weight per total volume in the solvent shown (Table I) unless otherwise stated. The preparation of glycine ethyl ester from the hydrochloride is described in the experimental section.

The podophyllin extract used was prepared from the crude resin by extraction with acetone. Dosage was calculated on the dry weight of the extract after filtration and evaporation.

Technique of application.

The hair was clipped from the whole back, from forelimbs to tail, before treatment and at intervals when necessary. Solutions were delivered from calibrated pipettes, care being taken that they spread as evenly as possible over the whole clipped area. A glass spreader was used for solutions which did not otherwise spread easily (e.g. glycine ethyl ester hydrochloride).

Recording of tumours.

Mice were examined throughout the course of croton oil treatment at weekly intervals, and all tumours of 1 mm. diameter and over recorded.

The groups of mice in which tumours appeared were kept after the end of croton oil treatment and examined at intervals for the development of malignant tumours. Many of these mice are still under observation.

Examination of mice for lung adenomas at post mortem.

The majority of mice were killed one week after the end of the standard course of croton oil treatment, and examined *post mortem*. The lungs were removed and the surface of each lobe carefully examined, in the fresh state, for the presence of pulmonary adenomata.

Histological examination.

Specimens of skin from mice additional to each group were removed under ether anaesthesia three days after one and/or two applications of the test substance. Mice which died during or were killed at the end of the tests were examined *post mortem* for lung adenomata and other abnormalities. Specimens for histological examination were fixed in Zenker's fluid, embedded in paraffin wax, and stained with haematoxylin and eosin-Biebrich scarlet (Gwynn and Salaman, 1953).

Experimental.

A. *Skin Tumour Production.*

Treatment with croton oil alone.

A group of mice (Group 43) were reserved as croton oil controls. They received weekly applications of 0·3 ml. 0·5 per cent croton oil in acetone. Altogether these mice have received 72 weekly applications, but for the purposes of controlling the present experiments only the first 18 applications need be considered, and for convenience we have called these " a standard course of croton oil treatment."

Tumour incidence.—Table I shows that one week after the 18th application of croton oil one of the 20 survivors bore 3 tumours. Subsequently tumours appeared at a steady rate, reaching the levels of 1 tumour per mouse in 19 survivors after 44 applications, 2 tumours per mouse in 17 survivors after 57 applications, and 3 tumours per mouse in 12 survivors after 66 applications. So far 3 malignant tumours have been seen in this group (after 55, 67, and 72 weekly applications respectively).

Class I : Antileukaemic agents.

Four substances in this class were tested : nitrogen mustard, Myleran, triethylene melamine (T.E.M.), and aminopterin. Table I gives details of treatment.

Groups 1 and 2 received applications of nitrogen mustard weekly for 15 weeks at dose-levels indicated by the results of a previously reported experiment (Salaman and Roe, 1953).

In a preliminary test single doses of Myleran and T.E.M. were given, which subsequently proved to be too high (Groups 3 and 6 respectively). A majority of the mice in both groups died, but the survivors were given the standard course of 18 weekly applications of croton oil.

TABLE I.—*The Screening of a Range of Substances for Initiating Activity.*

Group.	Number of mice.	Treatment with the test substance. Substance.	Total dose.	Method of application.†	Interval between 1st application of test substance and beginning of croton oil treatment.‡	Tumour incidence at the end of croton oil treatment. Tumour-bearing/surviving mice.	Tumours/surviving mice.
				CLASS I.—*Antileukaemic Agents.*			
1	10	Nitrogen mustard	1·5 mg.	15 weekly applications 0·03%	3 days*	3/10	4/10
2	10	„ „	4·5 „	15 „ „ 0·1%	3 „ *	1/9	1/9
3	24	Myleran	2·0 „	1 application, 0·67%	3 weeks	0/7	0/7
4	10	„	3·6 „	6 weekly applications, 0·067% and 4 weekly applications, 0·2%	4 days*	0/8	0/8
5	10	„	6·0 „	10 weekly applications, 0·2%	4 „ *	1/18	1/18
6	24	Triethylene melamine	0·5 „	1 application, 0·17%	3 weeks	3/7	7/7
7	10	„ „	0·24 „	1 „ 0·08%	3 „	5/10	18/10
8	10	„ „	1·8 „	15 weekly applications, 0·04%	3 days*	7/10	22/10
9	10	„ „	0·24 „	1 application, 0·08%	No croton oil treatment	0/8	0/8
10	10	„ „	1·8 „	15 weekly applications, 0·04%	„ „ „ „	0/9	0/9
11	25	Aminopterin	0·15 to 0·21 mg.	5 to 6 weekly applications, 0·01 to 0·02% in saturated solution of sodium bicarbonate in methanol (see text)	3 days*	1/14	1/14
				CLASS II.—*Substances Exerting a Specific Effect on Mitosis.*			
12	10	Colchicine	1·0 mg.	1 application, 0·33%	3 weeks	0/10	0/10
13	10	„	3·6 „	15 weekly applications, 0·08%	3 days*	1/8	1/8
14	25	Coumarin	45·0 „	1 application, 15%	3 weeks	1/17	2/17
15	40	„	150·0 „	1 application, 10%, followed at weekly intervals by 12 applications of 3·3%	10 days*	0/13	0/13
16	24	Hydroquinone	20·0 „	1 application, 6·7%	3 weeks	1/22	1/22
17	20	Podophyllin	4·5 „	15 weekly applications, 0·1%	3 days*	0/19	0/19
18	25	Sodium cacodylate	85·0 „	1 application, 28%, in methyl alcohol	3 weeks	0/19	0/19
				CLASS III.—*Narcotic Agents.*			
19	20	Carbromal	30·0 mg.	2 weekly applications, 5%	4 weeks	1/13	1/13
20	20	Chloral hydrate	24·0 „	2 „ „ 4%	4 „	4/17	4/17
21	20	„ „	225·0 „	15 „ „ 5%	3 days*	4/20	4/20
22	20	Chlorbutol	96·0 „	2 „ „ 16%	4 weeks	0/18	0/18
23	20	Methylsulphonal	48·0 „	2 „ „ 8%	4 „	2/18	4/18
24	20	Paraldehyde	300·0 „	2 „ „ 50%	4 „	1/18	1/18
25	20	Phenobarbitone	12·0 „	2 „ „ 2%	4 „	1/19	1/19
26	20	„	90·0 „	15 „ „ 2%	3 days*	1/20	1/20

Group.	Number of mice.	Treatment with the test substance. Substance.	Total dose.	Method of application.†	Interval between 1st application of test substance and beginning of croton oil treatment.‡	Tumour-bearing / surviving mice.	Tumours / surviving mice.
						Effect of croton oil treatment.	
		CLASS IV.—*Pharmacological Analogues of Urethane not Included in Classes I, II, and III.*					
27	20	o-Phenanthroline	300·0 mg.	10 weekly applications, 10%	3 days*	0/20	0/20
28	20	Physostigmine	2·25 „	15 „ „ 0·05%	3 „ *	0/17	0/17
		CLASS V.—*Urethane Derivatives and Related Compounds.*					
29	20	Benzocaine	1·2 g.	15 weekly applications, 27%	3 days*	0/19	0/19
30	20	Ethyl formate	2·76 g.	10 weekly applications of 0·3 ml. of the neat agent	3 „ *	0/14	0/14
31	18	Ethyl N-methyl carbamate	250·0 mg.	2 weekly applications, 42%	3 weeks	7/17	9/17
32	20	Ethyl N-octadecyl carbamate	180·0 „	9 weekly applications, 6·7%, in ether	3 days*	0/20	0/20
33	20	Ethyl N-phenyl carbamate	500·0 „	2 applications (with an interval of 15 mins.), 13·9% on 1st, 3rd, 5th, 8th, 10th, and 12th days	3 weeks	6/19	16/19
34	18	Ethyl thiolcarbamate	180·0 „	3 applications at intervals of 4 days, 20%	3 „	1/16	1/16
35	20	Glycine ethyl ester (for preparation see text)	0·57 g.	10 weekly applications, 19% in 20% aqueous carbowax 300	3 days*	0/18	0/18
36	20	Methyl carbamate	1·12 g.	15 weekly applications, 25%	3 „ *	1/18	1/18
		CLASS VI.—*Miscellaneous Substances.*					
37	20	1,2-Benzanthracene	6·0 mg.	2 weekly applications, 1%	3 weeks	7/18	21/18
38	10	„	6·0 „	2 „ „ 1%	No croton oil treatment	0/8	0/8
39	10	„	45·0 „	15 „ „ 1%	„ „ „ „	0/9	0/9
40	20	Cantharidin	0·63 „	9 weekly applications, 0·01%, followed by 6 weekly applications, 0·02%	17 days*	4/17	6/17
41	20	Indole	12·0 „	2 weekly applications, 2%	4 weeks	2/20	2/20
42	20	β-propiolactone	155·0 „	2 weekly applications, 10% and 5% respectively, followed, after an interval of two weeks, by 12 weekly applications, 2·5% (see text)	24 days*	15/19	160/19
		Control Group.					
43	20	Croton oil only (standard course‡)	—	—	—	1/20	3/20

* The earlier weekly applications of croton oil alternated at 3 to 4-day intervals with those of the test substance.

† Each application was 0·3 ml. of solution in acetone (unless otherwise stated) at the concentration shown (w/v in the case of solids, and v/v in the case of liquids.

‡ Croton oil treatment was standardized throughout at 18 applications of 0·3 ml. of 0·5 per cent solution in acetone.

Groups 4 and 5 were painted weekly with Myleran for 10 weeks, receiving altogether a higher total dose than mice in Group 3. As in all groups in which the test substance was given in divided doses at weekly intervals the first applications of croton oil alternated at 3 to 4 day intervals with those of the test substance.

Group 7 was given a single application of T.E.M., and Group 8 weekly applications of T.E.M. for 15 weeks. In both groups croton oil treatment was begun 3 days after the first application of T.E.M. When the standard 18 applications had been given it was decided to continue them for a few weeks, since at that time the number of tumours was increasing rapidly.

Groups 9 and 10 were given T.E.M. treatment corresponding to that of Groups 7 and 8 respectively, but no croton oil treatment thereafter.

Considerable difficulty was encountered in preliminary solubility and toxicity tests with aminopterin. The most suitable solvent for skin application was found to be a saturated solution of sodium bicarbonate in methyl alcohol (approximately 0·05 per cent). This solution was made up immediately before use. A single dose of 0·3 ml. 0·02 per cent aminopterin in this solvent was given to 20 mice in Group 11. This dose proved to be too high, and many died. Subsequently the survivors, together with 5 hitherto untreated mice, received 5 weekly applications of 0·3 ml. 0·01 per cent aminopterin in the same solvent. All mice began a standard course of croton oil treatment 3 days after the first application of aminopterin.

Tumour incidence.—The number of tumour-bearing mice, and the incidence of tumours in mice surviving until one week after the end of croton oil treatment, are shown in Table I. One week after the 18th application of croton oil 47 tumours were present in 27 survivors (Groups 6, 7, and 8) which had received treatment with T.E.M. and croton oil. This tumour incidence increased with further croton oil treatment. Fig. 2 shows the rates of development of tumours in these groups and, for comparison, that in Group 43 which received croton oil treatment only.

A significance test for the difference between the mean numbers of tumours per mouse in Groups 6 and 7 (pooled) and in Group 43, gives $t = 2{\cdot}90$ on 35 degrees of freedom, a value which could be exceeded by chance with a probability of $P = 0{\cdot}006$. The comparison between the mean numbers of tumours per mouse in Group 8 and in Group 43 gives $t = 2{\cdot}42$ on 28 d.f.; $P = 0{\cdot}02$. No tumours were observed in 17 survivors which received T.E.M. only (Groups 9 and 10).

It is concluded that T.E.M. is an effective initiator of skin carcinogenesis in the mouse, but not carcinogenic in the doses used.

Nitrogen mustard and croton oil (Groups 1 and 2) produced 5 tumours in 19 survivors at the end of croton oil treatment. This was in accordance with our previously reported borderline result with this substance (Salaman and Roe, 1953), the mean number of tumours being slightly greater than that obtained with croton oil only, but not significantly so ($t = 0{\cdot}57$ on 37 d.f.; $P = 0{\cdot}57$).

The distributions of the number of tumours on individual mice, in all the groups reported in this paper, are markedly skew. The significance levels indicated by the *t*-tests will be somewhat affected by this fact. Moreover it is possible that small differences in the incidence of tumours will be more readily detected by some quantity other than the mean number of tumours per mouse. For the comparison of Groups 1 and 2 (pooled) with Group 43, for instance, it is possible to rank together the mice in both series in order of number of tumours borne, and to ask whether the mean rank for Groups 1 and 2 (pooled) differs significantly from that

for Group 43. The appropriate test (Wilcoxon's) is described by Kruskal and Wallis (1952, pp. 590–592). Formulae for the expected value and variance of $\bar{R}$, the mean rank for mice in, say, the control group (it is immaterial which group is chosen), are given by Kruskal and Wallis. The square of the difference between $\bar{R}$ and its expected value, divided by its variance, is distributed approximately as χ^2 on 1 d.f. Application of this test to the series in question gives $\chi^2 = 1{\cdot}90$ on 1 d.f.; $P = 0{\cdot}16$. The difference is still not significant. However in some other groups, as shown below, the ranking test indicates a significant difference at the customary 5 per cent level, whereas the t-test does not.

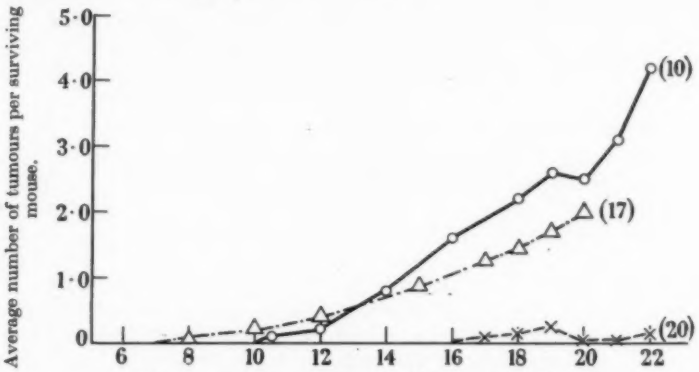


FIG. 2.—Initiating effect of triethylene melamine (T.E.M.).

△— · — · —△ Groups 6 and 7 (combined): 0·5 mg., and 0·24 mg. T.E.M. respectively (given as a single application in each case) followed by 20 weekly applications of of 0·5 per cent croton oil (beginning 3 weeks after treatment with T.E.M.).

○————○ Group 8: 1·8 mg. T.E.M. (15 weekly applications of 0·04 per cent) and 22 weekly applications of 0·5 per cent croton oil, alternating at first with the former.

×- - - - - - - - - × Group 43: Control group: weekly croton oil treatment only.

Acetone was the solvent throughout. The numbers of mice in each group at the beginning of the experiment and further details of treatment are shown in Table I. Numbers of survivors are shown in brackets.

Combinations of Myleran and croton oil (Groups 3 to 5) produced no more tumours than croton oil alone.

An attempt was made to give a higher total dose of Myleran. As in all experiments involving skin applications it is difficult to say how much of the apparent toxic effect of a substance is due to absorption from the gastro-intestinal tract following licking, and how much to absorption through the skin. To ascertain these proportions in the case of Myleran a group of 10 mice were prevented from licking themselves by means of plaster of Paris collars; 5 were then painted with 10 mg. Myleran to the clipped dorsal skin, and the remaining 5 mice similarly with 5 mg. Thereafter mice were kept in separate compartments to prevent their licking one another. The collars were removed after one week. All the mice which received 10 mg. Myleran died within 3 weeks, but those which received 5 mg. remained healthy. The latter were again put in plaster collars and given a

further application of 5 mg. each. Within 3 weeks of this second treatment all 5 were very sick or dead.

It was concluded that Myleran in the maximum tolerated dose was not an initiator of carcinogenesis in mouse skin, and that toleration could not be increased by the prevention of licking.

Mice treated with aminopterin and croton oil (Group 11) developed no tumours.

Class II : Substances exerting a specific effect on mitosis.

Five substances in this class were tested : colchicine, coumarin, hydroquinone, podophyllin, and sodium cacodylate. Details of treatment are given in Table I.

Tumour incidence.—In the doses employed none of these substances showed any evidence of initiating activity.

Class III : Narcotic agents.

Six substances were included in this class : carbromal, chloral hydrate, chlorbutol, methyl sulphonal, paraldehyde, and phenobarbitone. Details of treatment are given in Table I.

Tumour incidence.—In Group 20, which received two applications of 4 per cent chloral hydrate with a week's interval between them, followed by a standard course of croton oil, 4 tumours were present on 17 survivors one week after the end of croton oil treatment. The mean number of tumours does not differ significantly from that in Group 43 treated with croton oil only ($t = 0{\cdot}93$ on 35 d.f. ; $P = 0{\cdot}36$). The ranking test gives $\chi^2 = 2{\cdot}30$ on 1 d.f. ; $P = 0{\cdot}13$. The substance was re-tested at a higher dose (Group 21 : 15 weekly applications of 5 per cent). However in this group also the yield of tumours (4 in the 20 survivors) did not differ significantly from that in mice receiving croton oil only (Group 43).

In Group 25 which received 2 applications of 2 per cent phenobarbitone with an interval of one week between them, followed by a standard course of croton oil treatment, only one papilloma was present a week after the final application of croton oil. However a week later an ulcerated tumour appeared which was found, on histological examination, to be a fairly well differentiated squamous carcinoma which had penetrated the panniculus. Although an isolated finding this was, in our view, a remarkable one. We have never seen a malignant skin tumour arising spontaneously in mice of the strain used for these experiments. However, as described above, in Group 43, which received croton oil weekly for up to 72 weeks, 3 carcinomata have appeared, though none before the 55th week.

Because of this isolated finding phenobarbitone was re-tested at a higher dose level, in Group 26. However, no malignant tumours appeared in the group. It may be profitable to explore this part of the field again when we have learnt more of the process of initiation, and of the development of malignancy.

Class IV : Pharmacological analogues of urethane not included in Classes I, II and III.

Two substances in this class were tested : o-phenanthroline and physostigmine. Details of treatment are given in Table I.

Tumour incidence.—No tumours were seen in either group at the end of the standard course of croton oil treatment.

Class V : Urethane derivatives and related compounds.

Eight substances were included in this class : benzocaine (p-amino ethyl benzoate), ethyl formate, ethyl N-methyl carbamate, ethyl N-octadecyl carbamate, ethyl N-phenylcarbamate, ethyl thiolcarbamate, glycine ethyl ester, and methyl carbamate. Details of treatment are given in Table I.

The solution of glycine ethyl ester (Group 35) was made freshly each week immediately before use, by the following method. The solvent was first prepared by adding 1 volume of carbowax 300 to 4 volumes of distilled water. A 30 per cent w/v solution of glycine ethyl ester hydrochloride was then made up in this solvent. To this solution aqueous sodium hydroxide was added drop-wise until it was neutral to phenol red. Finally the volume was adjusted, by adding more of the prepared solvent, to make a 19 per cent w/v solution of glycine ethyl ester.

Tumour incidence.—Of 17 survivors which received 250 mg. ethyl N-methyl carbamate followed by a standard course of croton oil (Group 31) 7 bore a total of 9 tumours at the end of treatment. The difference between the mean number of tumours in Group 31 and that in Group 43, is not significant by the t-test ($t = 1{\cdot}57$ on 35 d.f. ; $P = 0{\cdot}12$). The ranking test, however, gives $\chi^2 = 6{\cdot}2$ on 1 d.f. ; $P = 0{\cdot}013$. In Group 33, which received 500 mg. of ethyl N-phenyl carbamate, of 19 mice surviving until the end of croton oil treatment 6 bore a total of 16 tumours. The difference between the mean number of tumours in Group 33 and that in Group 43 does not quite reach the customary level of significance by the t-test ($t = 1{\cdot}87$ on 37 d.f. ; $P = 0{\cdot}07$), but the ranking test gives $\chi^2 = 4{\cdot}3$ on 1 d.f. ; $P = 0{\cdot}04$. It must be emphasized that the ranking test was chosen after the data had been examined, and perhaps exaggerates the significance.

Fig. 3 shows the rates of development of tumours in Groups 31, 33, and 43, together with that in a group from a previous experiment (Salaman and Roe, 1953) which received a dose of urethane (240 mg.) approximately equivalent on a molar basis to those of the test substances in Groups 31 and 33.

We conclude that ethyl N-methyl carbamate and ethyl N-phenyl carbamate probably both possess some initiating activity, but of a lesser degree than that of urethane itself. Two further groups of mice are at present under test, both receiving ethyl N-phenyl carbamate at higher dosage than Group 33. One of these groups will receive no croton oil treatment, in order to determine whether the test substance is itself carcinogenic for mouse skin. (See Addendum II).

Ethyl thiolcarbamate (Group 34), given as three applications of 20 per cent w/v in acetone at intervals of 4 days, followed by croton oil treatment, gave rise to a solitary tumour in the 16 surviving mice. In a previously reported experiment (Roe and Salaman, 1954) a group of mice received three applications of 20 per cent w/v urethane in acetone at intervals of 4 days, followed by croton oil treatment ; in this group all of the 18 survivors bore tumours, and the total tumour yield was 70. We conclude that the substitution of a sulphur atom for the ester-linkage oxygen atom deprives the urethane molecule of its initiating activity.

Benzocaine, ethyl formate, glycine ethyl ester, and methyl carbamate, all applied in relatively high doses, showed no evidence of initiating activity.

Ethyl N-octadecyl carbamate in the dose given (see introduction) was without initiating effect.

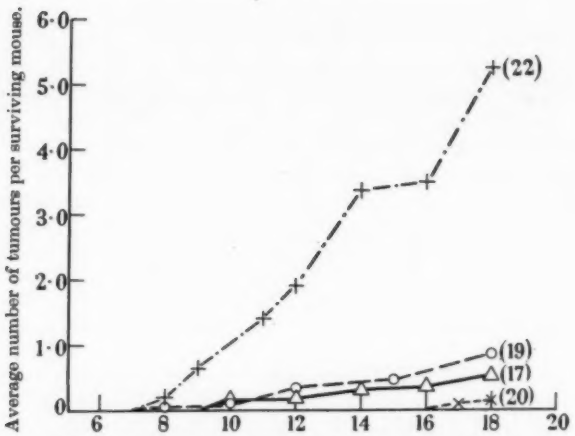


FIG. 3.—Initiating effect of urethane derivatives compared with that of urethane itself.

△————△ Group 31 : 250 mg. ethyl N-methyl carbamate (2 applications of 42 per cent with an interval of one week) followed by a standard course of croton oil treatment (18 applications of 0·5 per cent).

○— — — —○ Group 33 : 500 mg. ethyl N-phenyl carbamate (2 applications 13·9 per cent on 1st, 3rd, 5th, 8th, 10th, and 12th days) followed by a standard course of croton oil treatment.

+—·—·—+ A group from a previous experiment : 240 mg. urethane (2 applications 20 per cent w/v with an interval of 15 minutes, on 1st and 8th days) followed, 4 weeks after the 1st application of urethane, by a standard course of croton oil treatment.

×·····× Group 43 : Control group : weekly croton oil treatment only.

Acetone was the solvent throughout. The numbers of mice in Groups 31, 33, and 43 at the beginning of the experiment are shown in Table I. The group receiving urethane and croton oil consisted of 26 mice. Numbers of survivors at the end of the experiment are shown in brackets.

Class VI : Miscellaneous substances.

The four substances included in this class were 1,2-benzanthracene, cantharidin, indole, and β-propiolactone. Details of treatment are given in Table I. In the case of Group 37, painted with 1,2-benzanthracene and croton oil, treatment with the latter was continued for two weeks beyond the standard course, since at that time the number of tumours was increasing rapidly.

Tumour incidence.—In mice which received 1,2-benzanthracene and croton oil (Group 37), one week after the 18th application of the latter there were 7 tumour-bearing mice and a total of 21 tumours among the 18 survivors. At this stage the mean number of tumours differed significantly from that in Group 43 ($t = 2{\cdot}29$ on 36 d.f.; $P = 0{\cdot}03$). When two further applications of croton oil were given the incidence rose to 43 tumours in 13 mice out of 18 survivors. The rate of development of tumours is shown in Fig. 4. A control group which received similar doses of 1,2-benzanthracene without croton oil treatment developed no tumours (Group 38). Moreover no tumours appeared in a further group which were painted weekly with 1 per cent 1,2-benzanthracene for 15 weeks

(Group 39). We conclude that 1,2-benzanthracene is an initiator of carcinogenesis for mouse skin, but not carcinogenic in the doses given.

Of the 20 mice receiving applications of cantharidin together with a standard course of croton oil (Group 40) 17 survived until the end of treatment, and of these 4 bore a total of 6 tumours. The mean number of tumours does not differ significantly from that in Group 43 either by the t-test ($t = 0{\cdot}97$ on 37 d.f. ; $P = 0{\cdot}34$), or by the ranking test ($\chi^2 = 2{\cdot}45$ on 1 d.f. ; $P = 0{\cdot}12$).

Indole (Group 41) showed no evidence of initiating activity.

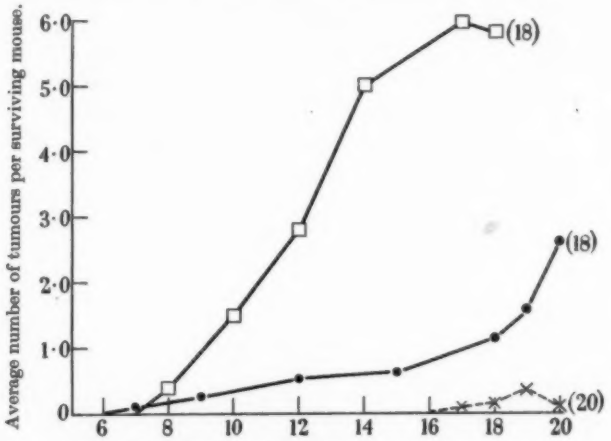


FIG. 4.—The initiating effect of 1,2-benzanthracene compared with that of 9,10-dimethyl-1,2-benzanthracene (DMBA).

●——● Group 37 : 6 mg. 1,2-benzanthracene (2 applications of 1 per cent with an interval of one week) followed by a standard course of croton oil treatment (18 applications of 0·5 per cent).

□——□ A group from a previous experiment : 0·3 mg. DMBA (single application 0·2 ml. 0·15 per cent) followed, after an interval of 4 weeks, by a standard course of croton oil treatment.

×·····× Group 43 : Control group : weekly croton oil treatment only.

Acetone was the solvent throughout. There were 20 mice in each group at the beginning of the experiment. Numbers of survivors are shown in brackets.

After the first application of 10 per cent β-propiolactone mice of Group 42 showed considerable ulceration and scabbing of the skin. The beginning of croton oil treatment was therefore postponed, and the concentration of β-propiolactone in the second application reduced to 5 per cent. However the inflammatory skin reaction persisted, and all treatment was withheld for a further fortnight. By then the skin lesions had healed, and weekly applications of 2·5 per cent β-propiolactone were begun. The skin reaction to this concentration was minimal. Weekly croton oil applications were begun 3 days later. Of 19 survivors one week after the final application of croton oil 15 bore a total of 160 tumours. The mean number of tumours differs significantly from that in Group 43 ($t = 3{\cdot}55$ on 37 d.f. ; $P = 0{\cdot}001$). The rate of development of tumours is shown in Fig. 5. In 3 mice

tumours appeared before the 6th application of croton oil, which was earlier than tumours normally appear in mice painted weekly with croton oil following treatment with an initiator. We are therefore led to suspect either that β-propiolactone is carcinogenic for mouse skin, or that the scarring resulting from the first two applications of β-propiolactone acted as an additional promoter of tumour formation. The substance is being tested again, both by itself and together with croton

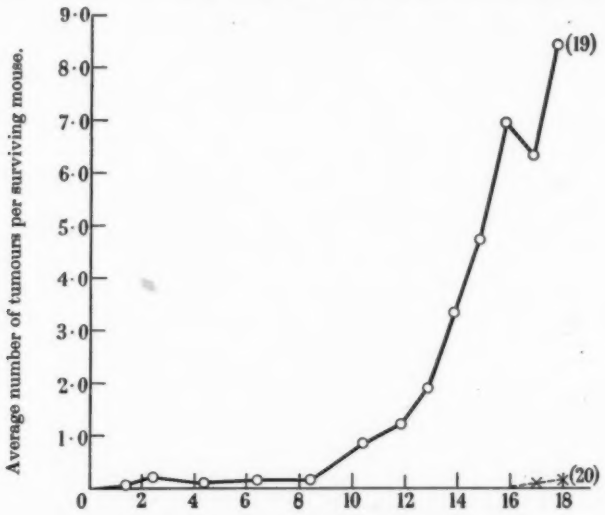


FIG. 5.—Tumour incidence in mice treated with β-propiolactone and croton oil.

○————○ Group 42: 155 mg. β-propiolactone (14 weekly applications, beginning at 10 per cent and falling to 2·5 per cent, see text) and 18 weekly applications of 0·5 per cent croton oil alternating at first with the former.

×- - - - - - - - - × Group 43: Control group: weekly croton oil treatment only.

Acetone was the solvent throughout. Both groups consisted of 20 mice at the beginning of the experiment. Numbers of survivors are shown in brackets.

Note.—In Group 42 tumours began to appear after only one week of croton oil treatment (i.e. 6 weeks earlier than in any other group).

oil. There is no doubt that β-propiolactone possesses initiating activity, but it is not yet possible to say whether in non-ulcerating doses it has promoting power as well. (See Addendum I.)

B. *Histological Findings in the Skin.*

The presence or absence of hyperplasia 3 days after the first and/or second applications of the test substances is shown in Table II.

Of the substances which, in conjunction with croton oil, gave rise to skin tumours, T.E.M., ethyl N-methyl carbamate, and ethyl N-phenyl carbamate, caused no hyperplasia of the mouse epidermis when applied alone. The reaction to β-propiolactone varied remarkably from mouse to mouse: one mouse three days

TABLE II.—*Histological Appearances in Mouse Skin after One or Two Weekly Applications of the Test Substances.*

Substance.	Concentration in acetone (per cent).	Day of biopsy.	Epidermal hyperplasia.
Nitrogen mustard	0·1	3rd and 10th	++
Myleran	0·67	,, ,, ,,	0
T.E.M.	0·17	,, ,, ,,	0
Aminopterin	0·02 (in saturated solution of $NaHCO_3$ in methyl alcohol)	,, ,, ,,	0
Colchicine	0·33	,, ,, ,,	+++
Coumarin	15·0	3rd	0
Hydroquinone	6·7	,,	0
Podophyllin	0·1	3rd and 10th	++
Sodium cacodylate	28·0 (in methyl alcohol)	3rd	±
Carbromal	5·0	10th	0
Chloral hydrate	4·0	,,	0
Chlorbutol	16·0	,,	0
Methyl sulphonal	8·0	,,	0
Paraldehyde	50·0	,,	0
Phenobarbitone	2·0	,,	0
o-Phenanthroline	10·0	3rd and 10th	+
Physostigmine	0·05	3rd	0
Benzocaine	27·0	3rd and 10th	0
Ethyl formate	100·0	,, ,, ,,	0
,, N-methyl carbamate	42·0	,, ,, ,,	0
,, N-octadecyl carbamate	6·7 (in ether)	3rd	0
,, N-phenyl carbamate	20·0	3rd and 10th	0
,, thiolcarbamate	20·0	3rd	0
Glycine ethyl ester	19·0 (in 20 per cent aqueous carbowax 300)	3rd and 10th	0
Methyl carbamate	25·0	,, ,, ,,	0
1,2-benzanthracene	1·0	,, ,, ,,	++
Cantharidin	0·01	,, ,, ,,	++
Indole	2·0	10th	0
β-propiolactone	2·5	3rd and 10th	0 to ++
Croton oil	0·5	,, ,, ,,	+++

after the 2nd application had an apparently normal epidermis, whilst others similarly treated showed quite marked hyperplasia. 1,2-Benzanthracene consistently gave rise to a moderate epidermal hyperplasia.

Of the substances which gave borderline results in the tests for initiation, cantharidin caused moderate epidermal hyperplasia. The reaction of the skin to nitrogen mustard has been described in a previous report (Salaman and Roe, 1953).

Both colchicine and podophyllin gave rise to striking histological appearances in mouse skin. In both cases the epidermis was thickened and many of the cells were greatly enlarged. Many clumped metaphases were seen, as well as aberrant chromosomes, fragmentation of chromatin, multinucleate cells, and a considerable number of pyknotic nuclei. These appearances have been fully described by other authors (King, 1948 ; King and Sullivan, 1946).

c. *Induction of Lung-adenomata.*

The experiments were not designed for the study of the induction of lung adenomata. Skin application would not have been the method of choice for that purpose. However, it is worth recording that the incidence of lung adenomata, as seen at *post mortem* one week after the end of croton oil treatment, was much greater in a few of the groups than in the rest.

Ethyl N-methyl carbamate followed by weekly croton oil gave rise to 26 adenomas in 9 mice out of 17 survivors, a result which confirms that of Larsen (1948) obtained by intraperitoneal injection of the substance alone. Methyl carbamate and croton oil gave rise to 12 adenomata in 7 mice out of 18 survivors; Larsen (1947*b*) failed to induce adenomata by intraperitoneal injection of this substance alone.

Ethyl formate and croton oil gave rise to 15 adenomata in 6 mice out of 13 survivors; the induction of lung adenomata by this substance has not been previously reported.

Ten mice treated with T.E.M. and croton oil (Groups 6, 7, and 8) have so far been examined *post mortem* at periods of 22 to 35 weeks after the end of croton oil treatment; the remainder are still under observation for the appearance of malignant skin tumours. Seven of the former bore a total of 63 lung adenomata. Although this result is not strictly comparable with those in the other groups, it suggests that treatment with T.E.M. followed by weekly croton oil produces a significant number of adenomata.

Of the mice painted with croton oil weekly for up to 72 weeks (i.e. 4 times the duration of croton oil treatment received by other groups), some of which are still under observation, 15 mice have so far been examined *post mortem*. Ten of these bore no adenomata, the remaining 5 bore 20, 3, 2, 1, and 1 respectively. It therefore appears that croton oil is capable of producing some lung adenomata, but at present the evidence depends mainly on the findings in a single mouse. It should be emphasised that this result cannot be used as a control for other groups because of the much longer period of croton oil treatment and greater age of the mice at the time of examination in this group.

Among the other groups still under observation for the development of malignancy (9, 10, 37, 38, 39, and 42) data on the incidence of lung adenomata is at present inadequate.

In all the remaining groups the mice were killed one week after the end of croton oil treatment. The total incidence was 45 adenomata in 394 mice examined, and did not exceed 1 adenoma per 4 survivors in any one group.

These results cannot be satisfactorily assessed until the rest of the mice still under observation have been examined *post mortem*, and until the incidence of lung adenomata in mice treated with the standard course of croton oil alone is known.

DISCUSSION.

As a sequel to the finding that urethane acts as an initiator of carcinogenesis for mouse skin (Salaman and Roe, 1953) the results of the screening of 29 substances for similar activity have been recorded. Many of these substances were selected for test because they possessed some other biological activity in

common with urethane, or could be regarded as structurally related to it. The reasons for selecting each particular substance are given in the introduction, together with a brief survey of the findings of other authors.

Three of the 29 substances tested in conjunction with croton oil by the methods described gave definite evidence of initiating activity, namely T.E.M., 1,2-benzanthracene, and β-propiolactone. Two of the derivatives of urethane tested, ethyl N-methyl carbamate and ethyl N-phenyl carbamate, each produced several tumours, but the differences between the mean number of tumours in either of these groups and that in the group treated with croton oil alone did not reach significance at the customary 5 per cent level when judged by the *t*-test. By a ranking test (Kruskal and Wallis, 1952),however, these differences were judged significant. The reason for using the latter test and the need for caution in accepting the results of its application have been given above. Three other substances, nitrogen mustard, chloral hydrate, and cantharidin, gave rise to a few more tumours than were seen in mice treated with croton oil alone, but the differences were not statistically significant by either the *t*-test or the ranking test. The remaining substances, which included Myleran, aminopterin, colchicine, podophyllin, and six narcotic agents, showed no evidence of initiating activity.

The base line against which initiating activity was measured was the tumour incidence in mice treated with croton oil only, for the same period as the test mice. Three papillomas developed in a group of 20 croton oil treated mice during this period (i.e. 18 weeks). But when applications were continued the incidence of tumours steadily increased until, at 72 weeks, an average of over three tumours per surviving mouse was reached. Moreover three malignant tumours appeared between the 13th and 17th months of treatment. Although precautions against contamination of the test mice with traces of carcinogenic hydrocarbons, or other initiators, from cages or fomites, or in the process of handling, were always taken, the possibility of such contamination cannot be excluded. Alternatively, it may be that croton oil, besides being the most powerful known promoting agent for mouse skin, has also initiating power of a low order. It must be remembered, however, that spontaneous skin tumours do occur in mice (Slye, Holmes and Wells, 1921) although we have not encountered them in the " S " strain used for these experiments ; and it is possible that latent tumour foci are present in normal mouse skin, which do not develop into tumours in the life-time of untreated mice, but may be induced to do so by prolonged treatment with croton oil.

The incidence of malignant tumours of the skin in mice treated with initiators and croton oil, and in mice treated with croton oil alone, is under study at present.

In the five groups in which initiating action is considered to have been demonstrated, not only did tumour incidence at the end of croton oil treatment exceed that of the croton-oil controls, but tumours appeared much earlier. Perhaps the most interesting findings is the initiating action of T.E.M. If total dosage is calculated on either a weight or a molar basis T.E.M. is far more effective than urethane. It is also far more toxic. Like urethane it does not give rise to skin tumours when applied repeatedly in maximum sublethal doses without croton oil, nor to any epidermal hyperplasia or other recognisable histological change. Moreover, like urethane it possesses antileukaemic, antimitotic, and lung adenoma-inducing action. The only striking differences in biological activity between the two substances are the hypnotic action of urethane, and the production of subcutaneous sarcomata by T.E.M. (Walpole *et al.*, 1954).

The findings in the case of Myleran are of interest since, though an anti-leukaemic agent, and a carcinogen in rats, it failed to initiate skin tumour formation or to produce lung adenomas in the mouse. In the last respect our findings confirm those of Shimkin (1954). In view of these negative results it seems desirable to confirm the carcinogenicity of this substance for the subcutis of the rat (Koller, 1953 ; 1954, personal communication).

Cornman (1954), discussing the activity of a series of carbamates, concludes that " almost any radical added to urethane increases its effectiveness as a mitotic poison and decreases or removes its carcinogenic action " (i.e. lung adenoma induction). Our results suggest that almost any modification of the urethane molecule is likely to decrease or remove the initiating activity for mouse skin. Moreover, none of the antimitotic substances in Class II (see Table I) of the present paper showed evidence of initiating activity.

Our findings in respect of lung adenoma induction by urethane derivatives agree in the main with those of Larsen (1947*a*, 1947*b*, 1948) who tested a wide range of these substances. However the rather high incidence in the group treated with methyl carbamate and croton oil is at variance with his findings. The fairly high incidence in mice treated with ethyl formate and croton oil is also of interest. Both these results require confirmation.

Among the test substances unrelated to urethane two showed initiating action. The finding that 1,2-benzanthracene is an initiator confirms the result of Graffi (1953). In view of the relatively small dose (6 mg.) required to initiate skin tumour formation it is possible that the low carcinogenicity of this substance when applied alone (see introduction) is due to its relative lack of promoting activity. This result suggests that it would be worthwhile testing other substances on the borderline of carcinogenicity for initiating action.

β-propiolactone is undoubtedly an initiator of carcinogenesis in mouse skin. However the fact that tumours appeared as early as the second week of croton oil treatment suggests that it may prove to be a carcinogen for mouse skin, as it is for the subcutaneous tissue of the rat (Walpole *et al.*, 1954). A further suggestion that this is so is given by the fact that 4 malignant tumours have already appeared among 19 surviving mice only 25 weeks after the beginning of treatment with β-propiolactone and croton oil. Malignant tumours have only appeared in other groups much later than this. It will not be possible to decide upon the status of this substance until further tests, now under way, are complete. (See Addendum I).

Table III shows the relation between the various biological activities (as far as they are known) of a number of the substances tested. There are many gaps, and some of the negative findings refer to experiments too limited to exclude a weak positive action. However it is perhaps permissible to suggest that there is some correlation between tumour-initiating activity for mouse skin, adenoma-inducing activity for mouse lung, and carcinogenicity for other species or tissues. But there seems to be no correlation between initiating activity and antimitotic or hypnotic activities, or between initiating activity and the ability to cause hyperplastic changes in the epidermis.

It is perhaps not surprising that substances which are capable of inducing tumours of the lung or other tissues in the mouse, or in other animals, act as initiators of carcinogenesis in mouse skin. Indeed the likelihood of this was the reason for selecting several of the substances. However the range of substances

TABLE III.—*The Relation Between the Ability to Initiate Tumour Formation in Mouse Skin and Certain Other Properties.*

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Substance.	Initiation of skin tumour formation (mouse).	Induction of lung adenoma-formation (mouse).	Carcinogenicity for tissues other than lung.	Anti-leukaemic action.	Specific effect on mitosis.[1]	Hypnotic action.	Production of epidermal hyperplasia (mouse).
Urethane	++	+++	—	+	+	+	0
T.E.M.	+++	++[2]	Sarcomas (S.C. rat)[3]	+	+	0	0
Nitrogen mustard (HN2)	±	±[4]	Various sites (mouse)[5]	+	+	0	++
R48	0[6]	0[6]	Various sites(rat)[7]	+	+	0	++[6]
Aminopterin	0	0[8]	—	+	+	0	0
Ethyl N-phenyl carbamate (phenylurethane)	+	0	—	0[9]	+	+[10]	0
Ethyl N-methyl carbamate (methylurethane)	+	+[11]	—	0[9]	—	+[10]	0
1,2-Benzanthracene	+	0 or ±[12]	Sarcomas (S.C. mouse)[13] and skin tumours (mouse)[14]	—	—	0	++
β-propiolactone	+++	—	Sarcomas (S.C. rat)[3] and ? skin tumours (mouse)[15]	—	—	0	0 to ++
Colchicine	0	0	Skin tumours (mouse)[16]	+[19]	+	0	+++
Myleran	0	0[17]	Sarcomas (S.C. rat)[18]	+	+	0	0

Notes.—Except where specified data are from this report, or unpublished observations in this laboratory.

[1] For references see " Selection of substances," p. 177, *et seq.*

[2] Shimkin (1954) also reported induction of lung adenomas by T.E.M.

[3] Walpole *et al.* (1954).

[4] Shimkin (1954), Heston *et al.* (1953), and Boyland and Horning (1949) all report induction of lung adenomata following injection of nitrogen mustard. We failed to do so by application of this substance to the skin.

[5] Boyland and Horning (1949), and Griffin *et al.* (1951).

[6] In mice treated with R48 and croton oil (Salaman and Roe, 1953) there was no evidence of initiation of skin tumours nor of induction of lung adenomas.

[7] Haddow and Horning (1950) reported an adrenal ganglioneuroma and a gastric carcinoma in a rat treated with R48. Koller (1953) refers to sarcomata induced in rats by subcutaneous injection of R48.

[8] Shimkin (1954) also failed to produce lung adenomata with aminopterin.

[9] Skipper and Bryan (1949) observed no depression of the white cell count in mice treated with either ethyl N-methyl carbamate or ethyl N-phenyl carbamate.

[10] Skipper *et al.* (1948).

[11] Our result confirms that of Larsen (1948).

[12] Shear and Leiter (1941) reported an increased incidence of lung adenomata in two mice 1 year after the subcutaneous injection of 5 mg. 1,2-benzanthracene but Andervont and Shimkin (1940) failed to induce adenomata by the intravenous injection of the substance. Our data is insufficient.

[13] Steiner and Falk (1951).

[14] Kennaway, 1930; Cook, 1933; Barry *et al.*, 1935; and Hill *et al.*, 1951.

[15] It is possible that β-propiolactone is carcinogenic for mouse skin (see p. 196).

[16] Harde (1939).

[17] Shimkin (1954) also failed to induce adenomata with Myleran.

[18] Koller (1953; 1954, personal communication).

[19] Dixon and Malden (1908) reported a transient leucopenia in several species following injection of colchicine. Kneedler (1945) induced a remission in a single case of acute myelogenous leukaemia, but a fatal relapse followed 13 months later.

so far tested is small, and the possibility is not excluded that initiators may be found which by themselves do not give rise to tumours in any tissue. Certainly the fact is established, and has, we think, practical as well as theoretical importance, that there exist substances which under certain conditions produce no tumours, nor any other recognised change, in a tissue, yet alter it in such a way that when a different stimulus is applied, perhaps much later (Roe and Salaman, 1954), benign and malignant tumours appear.

SUMMARY.

1. As a sequel to the demonstration that urethane applied to mouse skin followed by repeated applications of a *promoting agent* (croton oil) acts as an *initiator* of carcinogenesis, 29 substances, most of them related pharmacologically or chemically to urethane, have been screened for similar activity.

2. Of four *antileukaemic agents* tested, triethylene melamine (T.E.M.) was found to be an effective initiator of carcinogenesis but not carcinogenic, for mouse skin, in the doses tested ; nitrogen mustard, Myleran, and aminopterin, in maximum sublethal doses, showed no initiating activity.

3. Of five *substances exerting a specific effect on mitosis* (in addition to those included in the category of anti-leukaemic agents), none showed initiating activity.

4. Of six *narcotic agents* tested none showed unequivocal evidence of initiating activity.

5. Of eight *urethane derivatives and related compounds*, ethyl N-methyl carbamate and ethyl N-phenyl carbamate showed weak initiating activity. In neither case was this as strong as that of a molecularly equivalent dose of urethane. (See Addendum II).

6. Four *miscellaneous substances* were tested. Of these 1,2-benzanthracene was found to be an effective initiator of carcinogenesis but not carcinogenic, in the doses used ; and β-propiolactone was found to be an initiator of carcinogenesis. The latter is being tested for carcinogenicity at present. (See Addendum I.)

7. The use of the "t" test of statistical significance for skew distributions, such as those encountered in these results, is discussed. As an alternative, a ranking test is proposed.

8. The histological appearance of the skin following one, or two, applications of the test substances is described. Of those with initiating activity triethylene melamine, ethyl N-methyl carbamate and ethyl N-phenyl carbamate gave rise to no recognisable changes in the skin. 1,2-Benzanthracene consistently produced a moderate epidermal hyperplasia. The response to β-propiolactone varied from slight to marked epidermal hyperplasia.

9. The results are discussed, and an attempt is made to correlate initiating activity with other properties.

We are indebted to Dr. P. Armitage, of the Statistical Research Unit, London School of Hygiene and Tropical Medicine, for valuable assistance in the mathematical treatment of the results, and in particular for suggesting the use of the ranking test for significance.

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ADDENDUM

Since this paper was submitted for publication some of the experiments refered to in the text as still in progress have been completed.

I: *Further observations on β-propiolactone.* (cf. pp. 194, 198, 200.)

a. A group of 10 mice painted weekly for more than 20 weeks with a sub-ulcerative concentration (0·3 ml. 2·5 per cent in acetone) of β-propiolactone has so far exhibited no skin tumours. In another group which received 10 similar applications of β-propiolactone together with a standard course of croton oil treatment, 108 papillomas were present on 7 of the 8 survivors at the end of treatment. A further group received a single application of 2·5 per cent β-propiolactone followed after an interval of 3 weeks by a standard course of croton oil. Five of the 9 survivors in this group had a total of 22 papillomas at the end of treatment.

b. At *post mortem* only 1 lung adenoma was seen in 18 mice treated with β-propiolactone and croton oil.

Conclusion: There is no evidence that β-propiolactone applied in sub-ulcerative concentration is carcinogenic for mouse skin, but its initiating action has been confirmed.

II: *Further observations on ethyl N-phenylcarbamate (phenylurethane).* (cf. pp. 191, 200.)

Twenty mice received 120 mg. phenylurethane (2 applications 20 per cent w/v in acetone with 15 minutes interval) weekly for 7 weeks, and 60 mg. weekly for a further 8 weeks. Three days after the first application of phenylurethane a standard course of croton oil was begun. At the end of treatment only 1 of the 16 survivors bore tumours (5 papillomas), an incidence not differing significantly from that in mice receiving croton oil only.

A further group which received similar treatment with phenylurethane but no croton oil developed no skin tumours.

Conclusion: This test has failed to confirm the suggestion that phenylurethane possesses weak initiating activity, and has shown that it is not carcinogenic for mouse skin in the doses tested.

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THE INHIBITORY EFFECT OF ACENAPHTHENEQUINONE-BISULPHITE UPON TUMOUR GROWTH IN MICE.

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QUINONES have been shown to be biologically active components by many workers. They have been reported to act as mitotic poisons on fibroblasts grown in tissue culture (Meier and Allgöwer, 1945; Meier and Schär, 1947) and on embryonic cells of the freshwater annelid *Tubifex* (Lehmann, 1942, 1945; Huber, 1945, 1947). Mitchell and Simon-Reuss (1947) described the inhibitory effect of tetrasodium 2-methyl-1 : 4-naphthohydroquinone ("Synkavit") on normal and malignant cells grown *in vitro*. Friedmann, Marrian and Simon-Reuss (1948) have studied the antimitotic activity of 1 : 4-naphthohydroquinone diphosphate and its 2-methyl homologue on cultures of chick fibroblasts *in vitro*. The former was found to be 1000 times as active as the latter. Mitchell (1948) also reported increased radiosensitivity in tumours of patients concurrently treated with "Synkavit" and has recently (1954) reviewed later researches on this and related compounds. It was subsequently claimed by Gellhorn and Gagliano (1950) that "Synkavit" had no appreciable inhibitory effect on the growth of transplantable tumours in mice and rabbits. Quinones have considerable bacteriostatic powers (Hoffmann-Ostenhof, 1947). 9 : 10-Phenanthraquinone has a definite inhibitory effect on tumour growth in mice (Powell, 1951).

The narcotic urethane has been reported by Lasnitzski (1949) and Hughes (1950) to inhibit mitosis and by Haddow and Sexton (1946) to inhibit tumour growth in experimental animals. Acenaphthene-quinone, administered parenterally as the water-soluble sodium bisulphite additive compound, has a marked narcotic effect on experimental animals (Powell, 1949). This possession of narcotic properties by a quinonoid compound gave reason to investigate its effects upon tumour growth.

MATERIALS AND METHODS.

The transplantable tumours used in this study were Carcinoma 63 in A strain mice, Sarcoma 37 in R.III and A strain mice, and the following homologous tumours: a spindle-celled sarcoma and a squamous cell carcinoma in CBA strain mice (MV8), a polymorphic sarcoma (F2/52) and a squamous cell carcinoma in R.III strain mice.

Acenaphthenequinone-bisulphite in aqueous solution was administered by intraperitoneal or subcutaneous injection. Injections were made once daily for either the duration of an experiment (Experiments 1, 2) or for five consecutive days (Experiments 3–10) when the mice were concurrently irradiated. The dosage of the drug was adjusted to the body weight of each mouse. The drug solution was prepared by saturating 1 per cent sodium metabisulphite in aqueous

solution with purified acenaphthenequinone. This solution contained approximately 1·25 per cent acenaphthenequinone-bisulphite and about 0·5 per cent excess sodium bisulphite which served to stabilise the preparation. The acenaphthenequinone-bisulphite content of each preparation was estimated on the basis of the weight of acenaphthenequinone precipitated by sodium carbonate from an aliquot of the solution.

All the mice used in each experiment were inoculated subcutaneously with tissue from the cortex of an actively growing parent tumour. Treatment began when the transplants were about 24 mm.^{2} in area. The tumours were measured twice weekly with calipers and the surface area taken as the product of the major and minor axes. Inhibition of tumour growth was estimated as the percentage difference between the increase in area of the tumours of the control and treated animals from the beginning of treatment. Although this method does not give an absolute measure of tumour growth, it is adequate for assessing relative inhibition of growth in comparable experimental conditions.

X-rays were administered by an 88 kV. Siemens Roentgen camera delivering 96 ± 4 r/min. at 3mA. At each irradiation all tumours were treated with half the total dosage from each of two opposing directions, the centre of a tumour being placed at the focus of the X-ray beam 10·5 cm. from the aperture. No filters were used. Lead body shields protected the mice from scattered radiation. In groups of mice treated with both X-rays and acenaphthenequinone, the tumours were irradiated approximately 5 minutes after the drug was injected. The total dose was split into 5 equal doses administered every 24 hours to give a greater time in which the drug and X-rays might act in combination. In the experiments described, which are typical in each instance for each set of conditions, each group contained 10 mice except Experiments 1 and 2, in which 22 mice were used in each group.

EXPERIMENTAL RESULTS.

Dosage of acenaphthenequinone-bisulphite and anaesthetic effect.

The figures given are in terms of mg. acenaphthenequinone-bisulphite in an approximate 1·25 per cent solution administered by intraperitoneal injection. For mice of about 25 g. body weight the lethal dose was found to be 12 ± 2 mg. in R.III and CBA. strain mice, and 10 ± 2 mg. in A strain mice. Mice of the last strain appear in general to be more susceptible to toxic chemical substances than mice of the former two strains.

The drug had a marked narcotic effect. The duration of anaesthesia varied considerably with individual mice; for mice of 25 ± 3 g. body weight receiving a dose of 6 mg./20 g. body weight, the duration was 200 ± 70 minutes at 8° C., 110 ± 40 minutes at 18° C. and 23 ± 3 minutes at 30° C. The minimum anaesthetic dose was found to be $3{\cdot}5 \pm 0{\cdot}5$ mg. for a mouse of 25 g. body weight. Deep anaesthesia was induced by dosages of 2 mg. above this level; the muscles relaxed, respiration was regular, fast and shallow. With sublethal dosages the skin became cold and irregular gasping occurred.

Effects of acenaphthenequinone-bisulphite on tumour growth.

The results are listed in Table I. The duration of each experiment is given from the beginning of treatment until the final tumour measurement.

TABLE I.—*Inhibition of Growth of Transplanted Tumours in Mice by Acenaphthenequinone-bisulphite and X-irradiation.*

Experiment.	Mg. drug per 20 g. body weight.	Duration of experiment in days.	Tumour.	Strain of mice.	Average growth of control tumours in mm.².	Percentage inhibition of tumour growth.		
						X-irradiation alone.	X-irradiation and drug.	Drug alone.
1	12	11	Sq. cell	R. III	123	—	—	39
2	7	11	„ „	„	115	—	—	32
3	7	7	„ „	„	90	30	61	48
4	4·7	8	„ „	„	84	16	52	18
5	7	7	S. 37	„	148	14	36	42
6	5	6	„	A	90	10	38	6
7	5	8	Sp. cell	CBA	77	−1	33	−1
8	5	11	MV8	„	104	−1	54	3
9	5	14	C.63	A	135	7	59	—
10	5	14	F2/52	R. III	128	2	37	—

Administration of the drug alone by either the subcutaneous (Experiment 1) or the intraperitoneal (Experiment 2) route gave tumour growth inhibitions of 39 per cent and 32 per cent, respectively, on the homologous squamous cell carcinoma in R.III strain mice. Larger dosages of the drug could be given by subcutaneous than intraperitoneal injection; but it was observed that some of the additive compound hydrolysed to liberate acenaphthenequinone at the site of injection in the former instance. The inhibitory effect of the drug was found to be of the same order with both methods of administration. In view of the difference in drug dosages in these experiments this suggests that the quinone itself is not readily absorbed. Acenaphthenequinone is only very slightly soluble in most aqueous media, and it is probable that injected additive bisulphite compound is widely dispersed in the animal before hydrolysis is complete.

The combined effect of X-rays and the drug upon the growth of similar tumours (Experiments 3 and 4) was greater than that given by either agent alone. Whereas the combined effect of 250 r and 7 mg/20 g. body-weight dosage of drug was less than the added effects of the two agents separately administered (Experiment 3), the converse result was obtained with a lower drug dosage (Experiment 4). A comparable relation was observed in trials of the combined agents upon Sarcoma 37 (Experiments 5 and 6).

The effect of X-rays and a low drug dosage acting together on homologous spindle-celled sarcomas (Experiment 7) and squamous cell carcinomas (Experiment 8) in CBA strain mice was found to be disproportionately great in comparison with the effects of these agents administered singly. Acenaphthenequinone-bisulphite at a similar dosage also significantly intensified the effect of 250 r on the growth of Carcinoma 63 and Sarcoma F2/52 (Experiments 9 and 10). Sodium bisulphite at concentrations present in the acenaphthenequinone-bisulphite solutions used was not found to have a significant effect on tumour growth.

DISCUSSION.

From these results it appears that in certain conditions the inhibitory effect of a combined treatment with X-irradiation and acenaphthenequinone-bisulphite upon the growth of transplanted tumours may be significantly greater than would

be expected if the result of the double treatment were a simple summation of the effects of the two agents acting independently. This augmented combined effect may not be manifested if either the X-irradiation or the drug dosage exceeds certain limits.

The mechanism of this combined effect on tumour growth is not understood, although the fact that the irradiation was carried out while the mice were narcotised by the drug is probably relevant. With a total dosage of 250 r and either a low or a high dosage of the drug, the combined inhibitory effect was approximately of the same order (Experiments 3 and 4 ; 5 and 6).

These results may be explained on the assumption that X-irradiation activates radicals in the substrate affected by the drug, resulting in more reactive groups being made available. Alternatively, the acenaphthenequinone-bisulphite might make tumour cells more sensitive to injury by X-rays. Evidence supporting this interpretation is that quinones can act as hydrogen-acceptors and mediators in redox systems ; the induced narcosis could affect oxygen concentration in tumours by diminishing oxygen consumption ; and Gray *et al.* (1954) have shown that increased oxygen concentration does increase tumour radiosensitivity.

It should be emphasised that in almost all instances the treatment given was not curative ; the inhibition of tumour growth was only partial and treated tumours tended to recover from the injurious effects after the cessation of treatment. In view of the possible value of methods of radio-sensitisation, the quantitive relation between drug and X-irradiation dosages and the mechanism underlying the combined effect of the two agents are being further investigated.

SUMMARY.

1. The effects of acenaphthenequinone-bisulphite, alone and in conjunction with X-irradiation, upon the growth of various transplantable mouse tumours have been studied.

2. Acenaphthenequinone-bisulphite had a marked narcotic effect on mice.

3. Acenaphthenequinone-bisulphite administered parenterally significantly inhibited the growth of transplanted tumours.

4. The inhibition of tumour growth produced by a combined treatment with 250 r X-irradiation, delivered in split dosages of 50 r at daily intervals, and acenaphthenequinone-bisulphite administered concurrently, was disproportionately greater than the simple summation of the effects of the two agents acting independently.

5. The induction of the combined inhibitory effect on tumour growth of X-irradiation and acenaphthenequinone-bisulphite is briefly discussed.

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THE ANTIGENIC STRUCTURE AND GENETIC BEHAVIOUR OF A TRANSPLANTED LEUKOSIS.

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PIONEER workers with transplanted tumours observed that the proportion of successful grafts tended to increase with successive transfers. A possible explanation of this phenomenon was suggested by genetical data. Strong (1926*a*, 1926*b*) was the first to show that tumours might mutate to give genetically simpler variants. Thus a mammary carcinoma that originally required 6–8 genes for successful transfer gave rise to a mutant requiring only one factor, and another that appeared to lose all strain specificity. Similar observations have been made many times since, as can be seen in the reviews of Bittner (1935) and Snell (1953).

It is now generally accepted that the genes concerned determine antigenic structure and it appears a logical deduction that these mutations result in some type of antigenic simplification. However it has long been clear that a genetic experiment does not give a completely accurate picture of antigenic complexity. There are many reasons for this statement (Gorer, 1938), and here we need only give two examples. The most important antigenic system in tissue transplantation in mice is called the H–2 system. It has at least four antigenic components inherited as a bloc in a manner similar to the human Rh system (Hoecker, Counce and Smith 1954; Gorer and Mikulska, unpublished). A tumour studied by Gorer, Lyman and Snell (1948) gave a single gene ratio in a backcross experiment in which the animals had been typed for an H–2 antigen. Of 31 compatible for the antigen 28 died and 3 survived, whilst of 38 incompatible only 1 died. The 3 survivors in the former group did not appear to have been wrongly classified, and the authors concluded that the tumour must contain some minor antigens to which the host might respond under exceptionally favourable circumstances. On the other hand animals incompatible for H–2 may sometimes fail to respond and succumb to the tumour. However, this does not appear to be due to any modification of the H–2 antigens, since a tumour carried through a foreign strain may still elicit high titres of antibody (Gorer, 1950).

It seems clear that neoplastic cells may override antigenic differences, particularly those not belonging to the H–2 system, and it is of considerable interest to know how many of these minor antigens a genetically "simple" tumour may possess. If the number were to prove large it would show that mutations in tumours did not involve any antigenic loss, although of course it would not exclude the possibility that the relative proportion of various antigens is drastically altered (Gorer, 1948).

A direct serological study of the minor antigens is not yet feasible, and we therefore decided to study the effect of preliminary immunisation on the result of a genetical experiment.

MATERIALS AND METHODS.

The neoplasm used is a lymphogenous leukosis arising in the C57Bl. strain of mice known as E.L.4 (Gorer, 1950; Révész and Klein, 1954). It is a highly virulent neoplasm, and inoculated by the intraperitoneal route it may give rise to a fatal malignant ascites in mice of any strain. Subcutaneous inoculation gives rise to a tumour at the inoculation site in all mice tested, in foreign strains this usually regresses in from 10–14 days.

Crosses were made between C57Bl. and strains A and Balb.C. The F_1 animals are of course susceptible, but survive from 18 to 21 days following intraperitoneal inoculation, whereas the C57Bl. survive about 14 days. The F_1 (A × C57Bl.) was crossed to the A strain to give the "A-backcross". The F_1 (Balb.C × C57) was crossed with Balb.C to give the "Balb.C. backcross" and bred *inter se* to give the "Balb.C F_2".

Our C57Bl. mice have the antigenic combination known as H–2b. Two of the components are detectable by the haemagglutination technique of Gorer and Mikulska (1954). One component called "B" appears to be unique to H–2b, the other antigen "E" is present in a number of H–2 combinations, including that of the A strain (H–2a). Therefore in the A-backcross one can only type for antigen "B". In the crosses with Balb.C (H–2d) one can type for "B" and "E" although it is only essential to type for one component. In the tables we shall simply refer to the mice as H–2 compatible and incompatible.

For immunisation whole blood from C57Bl. was given by intraperitoneal inoculation. The doses administered will be given below.

RESULTS.

(1) *A back-cross experiments.*

In view of the known virulence of E.L.4 we thought it likely that we would obtain a certain number of deaths amongst H–2b negative mice in the A-backcross and that a weak antigenic stimulus, such as whole blood, would protect them but would not have any great influence on those compatible for these antigens. Accordingly 14 H–2 compatible and 20 incompatible mice were given 0·1 ml. of blood followed 7 days later by the subcutaneous inoculation of 175 thousand leukotic cells. Only one animal showed a persistent growth, and this had to be killed owing to a respiratory infection. We thought that some technical error had occurred and after 21 days gave a further challenge of 3·4 million cells. Every mouse developed a subcutaneous tumour which regressed in all but two H–2 compatible mice whose lesions ulcerated and which died on the 28th and 38th day respectively. The latter is considerably longer than an F_1 animal might be expected to live and cannot be considered really compatible. However this may be, 12 of the 14 H–2 compatible animals appeared to have a high degree of resistence.

In the second experiment we included seven (A × C57Bl.) F_1 mice to ensure that the cell suspension was fully viable. Sixteen H–2 positive and 12 H–2 negative A-backcross mice received no treatment prior to inoculation of the malignant cells, whilst 11 H–2 positive and 18 H–2 negative mice received 0·1 ml. of blood 12 days prior to the subcutaneous inoculation of 540 thousand leukotic cells.

Six of the seven F_1 controls developed tumours and all were dead by the 25th day. Amongst the unimmunised A-backcross mice the masses regressed in all those incompatible for H–2. Of the compatible mice 13 developed tumours, of which one regressed whilst the others were dead by the 25th day. Two failed to show any tumour. No lesions were seen amongst the immunised mice.

TABLE I.—*Comparison of Response to E.L.4 by Subcutaneous Inoculation in Immunised and Non-immunised A-backcross Mice.**

	Non-immunised.			Immunised.		
	Died.	Survived.	Total.	Died.	Survived.	Total.
H–2 Compatible . .	14	2	16	1	10	11
Incompatible . .	0	12	12	0	18	18
Totals . . .	14	14	28	1	28	29

* Survivors of the subcutaneous inoculation were given a further challenge—see text.

The result obtained with the unprotected A-backcross mice (12 positive, 16 negative) would be accepted as a good approximation to a 1 : 1 ratio in the absence of serological data. However, we were disturbed by the failure of one F_1 mouse and 2 H–2 positive A-backcross mice to develop any lesion, and gave a further intra-peritoneal challenge of 12·4 million cells 40 days after the first. The remaining F_1 control died on the 14th day. Of the unprotected A-backcross mice two more H–2 positive animals died whilst two survived. Both had apparently been immunised by the first subcutaneous inoculation. As can be seen from Table I the result of the double challenge (14 positive, 14 negative) is a perfect fit to a 1 : 1 ratio, and in the absence of serological typing we might have no suspicion of the underlying complexity. Amongst the immunised mice one H–2 positive animal died on the 14th day. No other mice in this group died although they were subsequently given a further challenge of 70 million cells. It is therefore apparent that 10 of 11 H–2 positive mice had developed a high degree of immunity.

For the third experiment we decided to give a single intraperitoneal challenge. We wished to know the mortality of unprotected H–2 positive and negative animals under these conditions, but felt that only H–2 compatible mice were of interest for their ability to be immunised. Therefore our control group consisted of 13 H–2 positive and 12 H–2 negative mice and the experimental group of 12 H–2 positive mice. The latter received 0·3 ml. of blood and 10 days later all were given an intraperitoneal dose of 142 thousand cells.

The result is shown in Table II in the form of a life table. It will be noticed that all the unprotected H–2 positive animals were dead by the 26th day, which is the type of result one would expect with F_1 mice. One H–2 negative mouse died on the 17th day, whilst 6 more died subsequent to the 29th day. If the experiment had terminated on the 26th day we should have obtained a good approximation to a 1 : 1 ratio (14 positive, 12 negative). Only one of the 12 H–2 negative mice showed minimal resistance, the other 6 which succumbed survived longer than the H–2 compatible mice and some developed atypical lesions. Only one H–2 compatible mouse died in the immunised group, and this survived till the 39th day and thus showed some evidence of immunity.

TABLE II.—*The Survival Times in Days of Unimmunised A-backcross Mice following Intraperitoneal Injection of* 142 *Thousand Leukaemic Cells.*

		Days of death.						
		15.	17–18.	19–22.	25–26.	Over 29.	Died.	Survived
		Number dying.						
Control	H–2 Compatible	1	8	3	1	–	13	0
	Incompatible	0	1	0	0	6	7	5
Total		1	9	3	1	6	20	5
Immunised H–2 compatible		0	0	0	0	1	1	11

(2) *The Balb.C backcross and Balb.C* F_2.

We were admittedly surprised at the results reported above and therefore sought confirmatory evidence in crosses with the Balb.C strain. As controls in the backcross experiment were included 14 H–2 positive and 14 H–2 negative mice. The immunised group of 24 H–2 positive and 15 negative mice received 0·1 ml. of blood 10 days before receiving 187 thousand cells by intraperitoneal inoculation. Table III shows a result similar to that shown in Table II. In the unprotected group all the H–2 compatible mice died by the 23rd day; however, 4 incompatible mice died within this period, and at 25 days there were 18 dead out of 25, whilst 5 more deaths occurred subsequent to the 25th day. None of the 39 mice in the immunised group died within the experimental period, and these were given further challenges in order to stimulate antibody production.

TABLE III.—*The Survival Times of Control and Immunised Balb.C Backcross and Balb.C* F_2 *Mice following Intraperitoneal Challenge.*

	Days of death.						
	14–15.	17–18.	19–21.	22–23.	'27 and over.	Died.	Survived.
	Number dying.						
Control backcross:							
H–2 Compatible	9	3	1	1	–	14	0
„ Incompatible	1	1	2	0	5	9	5
Total	10	4	3	1	5	23	5
Immunised backcross:							
H–2 Compatible	0	0	0	0	0	0	24
„ Incompatible	0	0	0	0	0	0	15
Total	0	0	0	0	0	0	39
Immunised F_2:							
H–2 Compatible	0	2	0	0	0*	2	18*
„ Incompatible	0	0	0	0	0	0	9
Total	0	2	0	0	0	2	27

* Some died following further dosage.

It would appear that the number of genes determining susceptibility in pretreated mice is large. In such circumstances an F_2 generation is advantageous. Our sample consisted of 20 H–2 positive and 9 negative individuals, all of which received 0·1 ml. of blood 6 days before an intraperitoneal inoculation of 77 thousand cells. Two H–2 compatible mice died with typical lesions on the 18th day. Six more deaths occurred following further dosage for antibody production, the first of these occurred on the 39th day and 5 more died at varying intervals up to the 12th week. All survivors were killed and bled out at the 14th week. None of the late deaths can be taken as indicative of full compatibility, and only 2 of 20 H–2 positive mice can be so counted. It so happens that this coincides with the expectation for 8 genes. In a backcross the figure is 1/256, which demonstrates the value of an F_2 in such a situation.

DISCUSSION.

It has been suggested that E.L.4, which has been transplanted since 1943, may have become incompatible with its line of origin. This is possible, but where it is believed to have occurred it has been found possible to immunise the line in question. We have made several attempts to do this without any success as yet. This of course does not disprove the contention, but it is doubtful if the observed results can be explained in this way. If E.L.4 is really incompatible with the C57Bl. it means that the former has an antigen absent in the latter, in which case whole blood should not be capable of immunising against this hypothetical substance. As a further check we have injected F_1 animals with C57 blood and found no effect.

In crosses with Balb.C we have definite serological evidence of subsidiary antigens in E.L.4. Before discussing the findings it is necessary to clarify some points of terminology. A genetical experiment cannot tell how many antigens there are in a given system such as H–2, but can give information as to the number of systems involved. Sera prepared in Balb.C against E.L.4 contain anti-B and anti-E, and occasionally an agglutinin reacting with some other system. Usually reactions given by the latter are too weak to give useful information; however, one sample gave very strong reactions with H–2 negative mice. When the Balb.C F_2 was tested with this, 28 out of 29 gave strongly positive reactions. If a serum were to contain antibodies against two systems 1 animal in 16 would be negative, whilst with three systems the figure is 1 in 64. The observed result is midway between and we did not have enough serum to use for absorption experiments, which should have clarified the situation. Sera from some of the H–2 compatible mice gave positive results. Most of them agglutinated A strain red cells with marked zoning, only the stronger samples agglutinated those of C57Bl. as well. This is not surprising; A strain red cells are far more sensitive to iso-agglutination than those of C57Bl. (Gorer and Mikulska, 1954) and the result may be taken to indicate the presence of at least one subsidiary antigen shared by these strains.

The only red cell agglutinin present in A anti-E.L.4 appears to be anti-B, although Amos (1953) has demonstrated additional white cell agglutinins which have not been studied genetically. Sera from H–2 positive A-backcross mice gave very weak reactions of doubtful significance, and serological evidence of subsidiary antigens was obtained indirectly. Samples of A anti-E.L.4 were

tested on several strains of mice by Dr. G. Hoecker whilst working at the Rosco Jackson Laboratory. He confirmed the presence of anti-B and in addition found weak reactions with considerable zoning in Snell's branch of the A strain. Both branches have the same H–2 antigens, and this must belong to some other system. Sera showing strong zoning may give negative results in heterozygotes, and this might well account for our failure to detect the antigen.

The A-backcross data are somewhat heterogeneous, but suggest that a smaller number of factors differentiates A and C57Bl. than is the case with Balb.C. Two out of 37 H–2 positive mice might be counted as fully susceptible, which suggests four or five factors in addition to H–2. However we do not wish to labour the exact number concerned since it is technically impossible to determine in experiments of this kind. Not only must very large samples be used to differentiate between various possibilities, but minor differences in the physiological state of the animals may influence their classification. All one can say here is that the results obtained with immunised mice are similar to those obtained by Bittner (1936) with splenic grafts. It therefore seems unlikely that any antigens have been lost, although their relative proportions may have been radically altered. The latter point appears to demand a direct serological approach, for which our technique is at present inadequate.

It is notoriously dangerous to generalise about neoplasms, and it remains to be seen how far E.L.4 may be an extreme case. Some preliminary results with A strain sarcoma have been far less striking than those obtained with E.L.4. However, it must be realised that whole blood is a poor antigen. It stimulates a low antibody response and does not accelerate the breakdown of skin homografts (unpublished work in conjunction with Billingham and Sparrow), and until more effective methods of immunisation have been studied, it will be unsafe to draw general conclusions from genetical data.

SUMMARY AND CONCLUSIONS.

Genetic data suggests that the increasing virulence shown by transplanted tumours may be due to antigenic simplification. However, tumours have been known to kill their host in spite of antigenic differences.

A highly virulent leukosis (E.L.4) arising in the C57Bl. strain of mice was tested in crosses with strains A and Balb.C. All animals were typed for their H–2 antigens. Some were challenged untreated, others received a preliminary dose of blood.

With unprotected mice, E.L.4 appeared genetically simple with the H–2 antigens playing a dominant role. In the case of protected mice it appeared highly complex. Some serological evidence of antigenic complexity was also found.

Whilst E.L.4 may be an extreme case, it would appear that virulence does not necessarily indicate any antigenic simplification.

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THE PERSISTENCE OF MOUSE ISO-ANTIBODIES *IN VIVO*.

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THE protection that can be demonstrated following the incubation of certain tumour cells with iso-immune sera *in vitro* is in striking contrast with the failure of the same sera to protect passively *in vivo* (see review by Hauschka, 1952), but before these findings can be interpreted it should be shown that the conditions obtaining in the body are as favourable for protection as those produced *in vitro*.

There are a number of factors that might be responsible for the failure of serum to protect, few of which have received sufficient attention. Foremost is the question of the activity of the serum, and of the relationship between this activity and the cell dose. Activity may either be measured in terms of protection or through some non-functional property, such as agglutinating power. The former would be too costly both in time and in animals for regular use, the measurement of the second has only recently been made simple and reliable following the introduction of the Dextran technique (Gorer and Mikulska, 1954). Consequently most of the work has been done on unstandardised serum. Similarly the cell dose has received little attention, although it is well known that a given volume of serum will only protect against a limited number of cells. Many experiments have been performed using a lumpy suspension or even solid fragments of tumour, in which case it is probable that serum cannot penetrate to the centre of the mass before the transplant is established. Under these circumstances it is not surprising that success has not been reported.

The object of this investigation was to provide a possible basis for a future attack on problems of passive immunity to tumours. During the course of the work it became apparent that the method would be useful for detecting antigens in genetical experiments and the second part of the paper is devoted to the results obtained.

MATERIALS AND METHODS.

The origin of the four pure line strains of mice, A, Bagg albino C (Balb.C), C_3H and C57 Black, together with a description of the C57 Black leukosis E.L.4, the production of antibodies, and details of the Dextran method of haemagglutination have already been published (Gorer and Mikulska, 1954).

After a few preliminary experiments in which the intravenous route was used, all antibodies were injected into the peritoneum. 0·1 ml. of serum was introduced into the peritoneal cavity through a fine-bore needle, care being taken to avoid leaking back. Equilibrium was reached in the bloodstream within an hour in homologous animals. When blood was required for testing, the animals were warmed to 37° C. before being anaesthetised with ether and bled from the tail. It was found that enough blood for titration could always be obtained

from a single mouse. The serum was removed from the clot and titrated immediately or stored in ampoules at — 20° C. for not more than 3 days. In some experiments designed to test for the persistence of antibodies, and in the genetical experiments, a single individual was bled; in the others, groups of three were bled simultaneously and the serum either pooled or titrated individually.

Backcross mice.—A backcross called the C backcross was obtained by mating Balb.C with C57 Black, and crossing the resultant F_1 with Balb.C.

Protection.—The simple technique already described (Gorer, 1942) has been followed. Fresh leukotic cells from ascitic fluid were suspended in normal mouse serum at a concentration of about one million cells in 0·1 ml. From this stock suspension a series of dilutions in normal serum was made covering the range required. An equal volume of immune serum was added and the mixture allowed to stand on the bench for 15–20 minutes and then inoculated subcutaneously into susceptible mice.

RESULTS.

Persistence of antibodies against E.L.4.

The antibodies studied here are those developed against the hist-compatibility system known as H-2 (Gorer, Lyman and Snell, 1948), which appears to be similar to the Rh system in man. Individual components are often shared between different strains, so that antibodies against E.L.4. produced in one strain will usually also agglutinate cells of other strains (Amos, 1953; Gorer and Mikulska, 1954; Hoecker, Counce and Smith, 1954); and antibodies may be detected by the use of several different red cells. In general, A strain cells are the most readily agglutinated and provide the most sensitive indicator of the presence of antibody.

After antibodies have been passaged through the homologous strain their agglutination reactions are similar to those of the original serum, but of lower titre owing to some degree of dilution. Thus Balb.C. anti E.L.4 may have an initial titre of 1/4000 for A cells and 1/2000 for C57Bl cells. After passage for 24 hours in Balb.C these values would be in the region of 1/256 for A cells and 1/128 for C57Bl., the titres falling slowly during the next 10 days to 1/16–1/32 and 1/4–1/16 respectively. Two such die-away curves are shown in Fig. 1. Here Balb.C anti E.L.4 has been injected into Balb.C, and C_3H anti E.L.4 into C_3H mice, the animals have been bled at intervals and the titres of circulating antibodies estimated against C57Bl. cells. For Balb.C anti E.L.4 the titre after 24 hours was 1/256 this level slowly falling to 1/16 after 8 days, the C_3H anti E.L.4 was a less active serum and from an initial level of 1/64 fell to 1/8 after the same interval.

The titre of circulating antibody after 18 hours is proportional to the amount of serum injected, for a range studied from 0·01 ml. to 0·2 ml. For 0·1 ml. of serum the dilution appears to be about 1/20. Assuming the blood volume to be 63·2 c.c. per kilo (Oakley and Warrack, 1940) and the haematocrit 43 per cent (Wintrobe, 1933) for mice of about 25 g. in weight, the serum volume would be approximately 0·7 ml. and would account for a dilution of about 1/8, so that rather more than half of the serum is being lost from the circulation, probably into the lymphatic and extracellular fluids.

In six separate experiments lasting from 8 to 12 days haemagglutinins prepared

against E.L.4 have been found to persist for a considerable time, and although there was too much variation between different samples of antibody for an accurate assessment of half-life, this was of the order of the value given by other workers (Dixon *et al.*, 1952). In some of the experiments there tended to be an initial period of up to 8 days in which the titre remained constant and then fell at about the usual rate. This lag was most noticeable when large amounts or exceptionally high titre sera were used, and was most marked with A strain red cells. Haemagglutination does not provide a good indicator for estimating half-life, but is a valuable method for detecting the continuing presence of antibody.

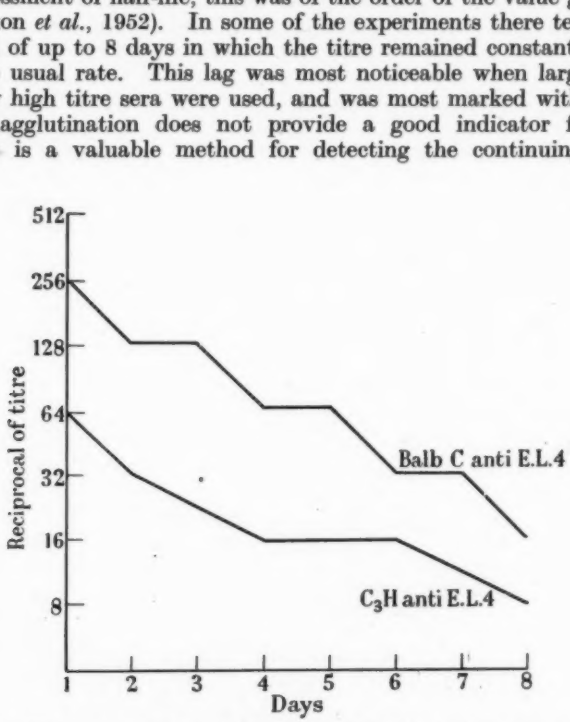

FIG. 1.—The persistence of antibodies against E.L.4 in the homologous strain; titrated against C57Bl. cells.

Protective antibodies also survive passage through the homologous strain. 0·2 of Balb.C anti E.L.4 was inoculated into some Balb.C mice which were bled out after 2 days. A protection experiment was then set up using 20,000 leukotic cells with 0·1 ml. of passaged antibody in the experimental group and 10,000 and 20,000 cells in normal serum for the controls. The control mice all developed tumours by the 13th day whilst 0·1 ml. of passaged antibody gave complete protection for 34 days, when the experiment was discontinued. A similar experiment in which a different serum was passaged for 3 days gave the same result.

The circulating antibody can also be absorbed by free leukaemic cells, but apparently not by cells outside the circulation. Three groups of Balb.C mice were taken, the control was given 0·1 ml. Balb.C anti E.L.4 alone, the first experimental group was given the same dose of antibody intraperitoneally followed almost immediately by 0·2 ml. of packed E.L.4 cells intravenously, the second was given the same dose of E.L.4 intravenously but the serum was only given

after 18 hours. Serum from the first group gave a titre of only 1/4 against C57Bl. cells, whilst for the controls and the second experimental group the titres were 1/32–1/64. Another experiment along similar lines was performed when homologous antibody was given to a group of six Balb.C mice that had actively growing tumours of E.L.4. The course of the transplanted leukosis in Balb.C is of progressive growth up to 7–8 days followed by rapid regression at about the 10th day, antibodies are not detectable in the blood until the tumour begins to regress. In this case the antibody titre in the animals with growing tumours followed closely the titre in a control group without tumours until the 9th day, when there was an abrupt rise in titre in the experimental group, so that whilst E.L.4 will absorb antibodies when freely circulating in the blood stream, it is unable to do so when established in the tissues. This failure to absorb by the growing tumours cannot be ascribed to saturation with locally produced antibody, as similar tumours absorb quite readily *in vitro*, but must be due to lack of intimate contact between the antibody and leukaemic cells. It can readily be seen that the use of tumour fragments could lead to the same result, probably purely a mechanical effect.

In vivo absorption.—In contradistinction to their prolonged survival in the homologous strain, passively transferred antibodies are quickly removed in the presence of the corresponding antigen. When 0·1 ml. of Balb.C anti E.L.4 was given intravenously to C57Bl. the titre against A cells after 5 minutes had fallen to 1/16, after 15 minutes to 1/4 and after 30 minutes could no longer be detected, the titre in Balb.C given the same dose remaining at 1/256. Even when the relatively enormous dose of 0·5 ml. of the same serum was given intravenously to the blacks the level after one hour was only 1/4, and after two hours the agglutinating antibodies had been completely absorbed.

This degree of absorption could not have been achieved by the red cells alone. Details of the antigenic structure of E.L.4 will be given by Gorer and Mikulska in a later paper, so that here it is sufficient to mention two of the antigens involved. The antigenic component H2–B is present in E.L.4 and in the tissues and red cells of C57Bl., H2–E is present in E.L.4 and the fixed tissues of C57Bl. is doubtfully present on the red cells ; it is however shared by other strains and is present on the red cells of the A strain. After repeated absorption with C57Bl. red cells Balb.C anti E.L.4 will still agglutinate A strain red cells, C57Bl. liver and E.L.4 will remove all the agglutinins. The removal of the antibodies by the blacks *in vivo* must therefore be due to contact with other cells, probably those of the bone-marrow and possibly by the cells of the liver and splenic sinusoids, etc.

TABLE I.—*Comparison between Absorption with Liver and* in vivo *Absorption of Antibodies against E.L.4.*

Antibody.	Tested on.	Titre* unabsorbed.	Titre* after absorption with liver from : A.	Titre* after absorption with liver from : Balb.C.	Titre* after passage through : A.	Titre* after passage through : Balb.C.
Balb.C anti-E.L.4	A cells	Over 2000	0	1024	0	256
	C57Bl. cells	Over 2000	256	1024	32–64	64
C_3H anti-E.L.4	A cells	256–2048	0	0	0	0
	C57Bl. cells	512–4000	256–512	256–512	64	32–64

* Titres expressed as reciprocals.

A comparison of some results obtained by *in vivo* and *in vitro* absorption is given in Table I. For the antibody Balb.C anti E.L.4, A strain liver removes all the antibodies against its own cells and lowers the titre against C57Bl. cells from over 1/2,000 to 1/256, a control absorption with Balb.C liver lowers the titre against both cells slightly. Figures for the *in vivo* absorption are similar, but the titres are lower. A similar situation occurs with C_3H anti E.L.4 where both A and Balb.C remove the agglutinins against A cells but not against C57Bl. Although there has been a considerable degree of dilution in the *in vivo* series the results are clear cut and there does not appear to have been any untoward degree of non-specific absorption. There is little point in using the technique to study the absorptive capacities of pure line strains, as liver is readily available; but in a genetical experiment the individual mouse may be valuable either for use in further experiments or for breeding. Any number of *in vivo* absorptions can be carried out provided they are adequately spaced.

TABLE II.—*Serological Typing of C backcross mice by direct Agglutination and by* in vivo *Absorption.*

	Direct agglutination :		*In vivo* absorbed serum tested on :	
Mouse No.	Balb.C anti-E.L.4.	A anti-E.L.4.	A cells Balb.C anti-E.L.4.	C57Bl. cells A anti-E.L.4.
17	+	−	+	+
20	+	+	−	−
21	+	+	−	−
23	−	−	+	+
29	+	+	−	−
36	−	−	+	+

In any serological typing of a genetical experiment there are always a few animals giving weak reactions. Some of these may be due to the serum used containing antibodies against subsidiary antigens (Amos, Gorer, Mikulska, 1955), simply repeating the direct agglutination with a different serum may serve to distinguish these. The classification of the others can only be decided by absorption. Red cell absorption is impracticable for removing " Dextran " antibodies, so that *in vivo* absorption is the method of choice. Table II shows some results of such an absorption with C backcross mice. The columns on the left show the results of direct agglutination with Balb.C anti E.L.4 and A anti E.L.4, the former detecting the presence of the antigens H2-B and H2-E, the latter H2-B. On the right is shown the result of testing the same two sera after passage through the backcross mice. Where the antigen is shown to be present on direct agglutination the corresponding antigen is removed by *in vivo* absorption. Mouse 17 gave a positive result with Balb.C anti-E.L.4 but not A anti-E.L.4 on direct testing, and this result was again obtained when the mouse was tested with a different serum. *In vivo* absorption showed that the H-2 antibodies were not absorbed, the reaction was due to some subsidiary antibody.

Although the technique is very straightforward, there are a few precautions that must be taken. Different sera must not be given at intervals of less than 3–4 weeks in order to allow the residual antibodies to decay and to allow the animal to make good the blood loss. Because animals used in a genetical experiment may have less of the antigen than the pure line it is advisable to allow 18

hours to elapse before bleeding. Finally, it is important to avoid sera from mice that have been too strongly immunised, as these may contain subsidiary antibodies which may give rise to difficulty in the interpretation of results.

DISCUSSION.

Gorer (1942) suggested that haemagglutinins and protective antibodies were distinct entities but this conclusion was based upon results with saline agglutination, which is an inefficient method of detecting antibodies and at that time the extreme complexity of the H-2 system was unknown. However, the statement may well be correct. Even if that is so, it is possible that the stability of protective antibody may be similar to that of the haemagglutinins. Experiments with Dr. Gorer indicate that their stability *in vitro* is similar and the protection experiments reported above indicate that this is also true *in vivo*. It would seem therefore that the decay of antibody is not too rapid to render passive immunisation impossible, but it would be unreasonable to expect success with cell dosages that are far beyond the power of a serum to inhibit when mixed *in vitro* prior to inoculation.

SUMMARY AND CONCLUSIONS.

When antibodies against a C57 Black leukosis E.L.4 are inoculated into non-immune homologous mice they may be detected in the bloodstream for as long as 12 days. Variations are too great for an estimate of the half-life to be made, but this appeared to be at least two days and in some cases longer.

Protective antibodies can also be detected in the blood of similarly treated animals for at least 3 days.

Passage through backcross mice results in absorption of antibodies by animals having the corresponding antigen.

My thanks are due to Dr. P. A. Gorer for helpful criticism and advice and to Mrs. Z. B. Mikulska for titrating some of the sera.

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PSEUDONEOPLASTIC NONSPECIFIC PROLIFERATIVE LESIONS ON THE CHORIOALLANTOIS OF THE CHICK EMBRYO.

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THE chorioallantoic membrane of the chick embryo is an actively growing embryonic tissue whose morphological reactions to a variety of stimuli are liable to be intense and sometimes bizarre. In the process of inoculating the membrane with virus-containing or other material, a certain degree of trauma is inevitable and a relatively well-defined sequence of lesions develops as a result.

The non-specific lesions developing within 2 to 4 days after innoculation have been described by Beveridge and Burnet (1946). They are of limited degree and as a rule do not give rise to serious difficulties of interpretation. However, with the use of longer periods of incubation, the further development of the lesions may give misleading appearances. In particular, there is a tendency for hyperplasia of both epithelial layers of the chorioallantoic membrane and the occurrence of clumps of epithelial cells in the mesodermal tissue lying between these layers. This combination may often give an appearance suggestive of neoplasia.

Reports have appeared in the literature describing similar pathological changes in the chorioallantois following innoculation of material derived from various tissues. Claims have been made for the specificity of these lesions and, in some cases, they have been taken as evidence for a viral agent being present in the tissue used as a source of the inoculated material.

In view of the increasing use of the embryonated egg in cancer research, a description of this type of non-specific lesion and some of the factors in its development seems desirable.

MATERIALS AND METHODS.

The embryonated eggs used in all experiments had been incubated at 37° C. for 11 days prior to inoculation.

Inoculation procedure.

The eggs were candled and the site of maximum development of the chorioallantois marked. At this site a hole, approximately 1 cm. in diameter, was drilled in the shell with a dental drill. A hole was also drilled in the air space. The exposed shell membrane was then cleared with a drop of liquid paraffin and the surrounding shell coated with a layer of melted paraffin wax.

A drop of normal saline was placed on each shell membrane.

Dropping of the chorioallantoic membranes was carried out by sucking over the air space with a rubber teat at the same time as a small perforation was made in the shell membrane by lateral pressure with a sterile needle.

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Inoculation followed immediately and the eggs were returned to a 37° C. incubator.

Material for inoculation.

Material used for inoculation included normal saline, nutrient broth and emulsified normal chorioallantoic membrane.

Where non-specific lesions were encountered whilst using inocula of the chorioallantoic membrane, passage of these lesions was performed. The affected chorioallantoic membrane was ground with sterile sand with pestle and mortar, and to this was added 5 ml. sterile broth with added penicillin and streptomycin.

The emulsion was centrifuged at 3500 r.p.m. for 5 minutes to remove the silica particles and larger cell debris.

The resultant supernatant fluid was used as the inoculum for a further batch of eggs.

The size of the inoculum was 0·05 ml. in each case.

Incubation procedure.

Following inoculation, the eggs were incubated in a horizontal position for one hour at 37° C. If eggs are incubated in this position for seven days with the hole in the overlying shell left open, considerable drying and atrophy of the membranes occur, making detection of lesions in the membranes difficult.

Therefore, after one hour, the eggs were removed from the incubator and the holes in the shell sealed with melted paraffin wax. The eggs were then placed vertically in racks, air-space uppermost, and returned to the 37° C. incubator for the remainder of the incubation period. This had the effect of allowing absorption of the air from the artificial air space and the dropped chorioallantois lined the shell membrane once more.

To eliminate the possibility that lesions observed on the membranes were due to specific effect of the paraffin used, parallel batches of eggs were inoculated without the use of paraffin. The holes in these eggs were sealed with plastic tape and the eggs incubated in a horizontal position.

Examination of membranes.

With the egg held horizontally, a cut with scissors was made, beginning at the air space and extending horizontally round the egg so as to divide the shell into two nearly equal halves. The chorioallantois lining the top half of the shell was peeled off the shell and spread out on a glass plate against a black background.

Lesions found were examined microscopically by placing the tissue in 10 per cent formol saline, embedding in paraffin wax, sectioning and staining with haematoxylin and eosin.

RESULTS.

Following the inoculation of the above mentioned materials, a variety of lesions was found on the chorioallantois when examined seven days later.

Macroscopic appearance of lesions.

Four main types of lesion occurred on the chorioallantoic membrane :

(*a*) Generalised oedema.

(*b*) A ring-shaped area of opacity and thickening of the membrane delineating the outer limit of the area of dropped membrane.

(*c*) Opacities along the blood vessels of the membrane.

(*d*) Discrete nodules in the membrane, single or multiple.

The first three of these types are also found in eggs incubated for only 2–3 days following inoculation and have been fully described by earlier workers (Goldsworthy and Moppett, 1935 ; Beveridge and Burnet, 1946).

The nodular type of lesion is only seen in eggs incubated for longer periods and is the type of lesion which is the subject of the present paper.

The number of membranes showing nodular lesions varies considerably from one batch of eggs to another. As may be seen from Table I, most batches had from 10–40 per cent of membranes showing lesions.

TABLE I.—*Incidence of Nodular Lesions in Egg Membranes.*

Experiment number.	Number of eggs used.	Number of eggs showing lesions.	Number of eggs dead.	Lesions. per cent
1	11	3	3	27
2	9	6	—	67
3	12	5	4	42
4	18	8	6	44
5	30	13	1	43
6	25	4	3	16
7	26	10	—	39
8	25	8	5	32
9	24	2	6	8
10	26	5	4	15
Total	206	64	32	31

The number of nodules per membrane was again variable. Most commonly, 1 to 3 nodules were formed, but occasionally as many as 10 were found.

Where single, the nodules were almost invariably found on that part of the membrane lying opposed to the original inoculation hole in the shell.

Where multiple, the nodules were found in the region of lumps of paraffin on the under surface of the shell or along the periphery of the dropped area of the membrane. More rarely, an apparently random distribution of nodules over the dropped area was found.

DESCRIPTION OF PLATES.

FIG. 1.—Chorioallantois. Nodule showing ulcer formation and chorionic and allantoic epithelial hyperplasia. Chorionic layer to left of figure. H. and E. × 130.

FIG. 2.—Chorioallantois. Nodule showing epithelial islands and granulation tissue. Chorionic layer to left of figure. H. and E. × 50.

FIG. 3.—Chorioallantois. Nodule showing numerous epithelial islands with a random distribution. Marked round cell infiltration. H. and E. × 75.

FIG. 4.—Chorioallantois. High power view of epithelial island. Note nuclear irregularity and cystoplasmic vacuolation. H. and E. × 765.

FIG. 5.—Chorioallantois. Mesodermal granulation tissue at base of ulcer. Note sarcomatous appearance. H. and E. × 680.

FIG. 6.—Chorioallantois. Allantoic epithelial layer showing well developed papillary formation. H. and E. × 170.

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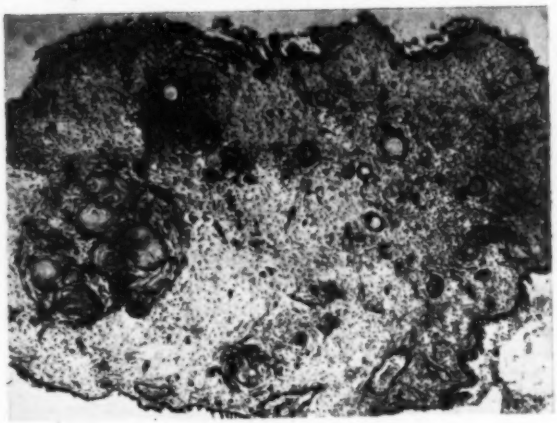

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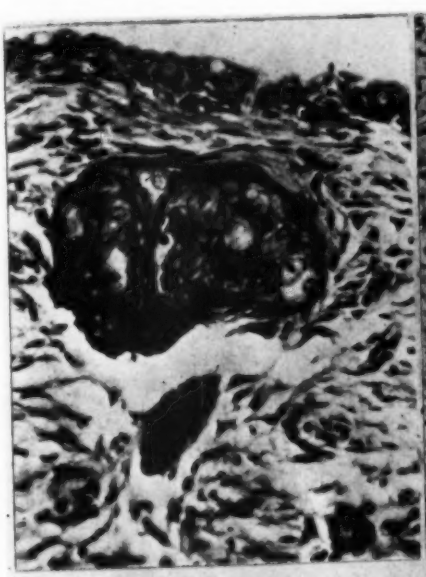

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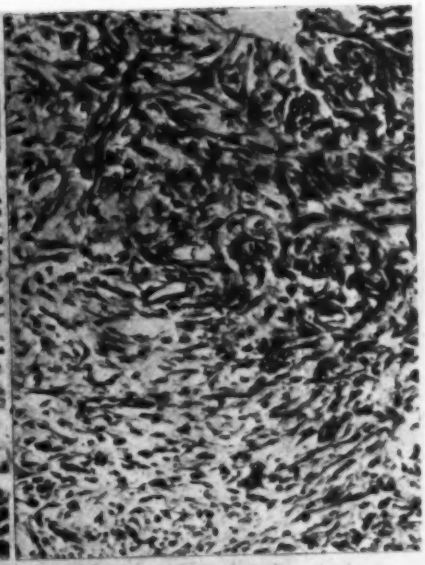

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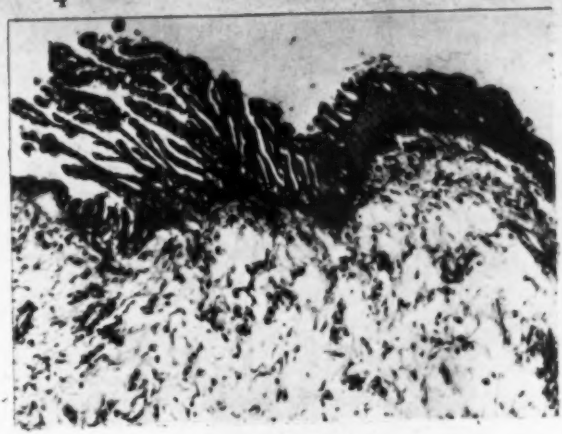

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The nodules varied considerably in size from a diameter of several millimetres to 1 cm.

The nodules were pearly grey in colour, opaque and rounded. They projected from the allantoic surface of the membrane and, when large, the central area of the nodule was sometimes ulcerated on the chorionic epithelium.

There was no halo of oedema around the periphery of the nodules and there was no obvious alteration in the vascular pattern of the membrane around the lesion.

Despite the use of penicillin and streptomycin in the inocula, fungal infections of the chorioallantois were sometimes found. In some cases, the lesions resulting had a nodular appearance, but they were easily distinguishable from the above nodules on the following criteria :

(*a*) Marked ulceration of the nodules with yellowish-golden exudate.

(*b*) Presence of mycelia on the surface of the membrane.

(*c*) Oedema of the surrounding membrane with striking radially arranged pattern of blood vessels.

Microscopic appearance of lesions.

On sectioning, the nodules were seen to contain abnormalities in all three layers of the membrane. Each nodule was basically an ulcer in which excessive regeneration of the ulcerated chorionic epithelium had occurred with a marked reaction in the underlying mesodermal tissue and a hyperplasia of the opposing allantoic epithelium (Fig. 1).

Frequently, the chorionic epithelium was absent over the nodule. However, the epithelium on either side of the nodule showed hyperplasia with an increase in thickness of the cell layer from a normal 2 or 3 cells to as many as 15 cells thick. In other nodules the epithelial layer had completely regenerated (Fig. 2) and the nodule was then covered by a complete chorionic layer.

Considerable variation was found in the relative size of the nucleus to cytoplasm of the cells and many cells showed intense basophilic staining of the cytoplasm.

Characteristic of the nodular lesions was the development of islands of chorionic epithelial cells in the underlying mesoderm (Fig. 3, 4).

In some nodules (Fig. 3), there was a random distribution of such islands throughout the nodule but, more commonly, they were found to form a chain extending from one side of the nodule to the other (Fig. 2).

The islands appeared to develop as a result of scattered groups of epithelial cells being isolated during the original ulcerative process in the chorionic epithelium.

During the healing stage of the ulcer, these epithelial islands were by-passed by the actively growing mesodermal granulation tissue. Consequently, when the chorionic epithelial layer was reconstituted by proliferation of the cells at the edge of the ulcer, the cell islands were isolated within the mesodermal tissue.

Occasional sections showed a continuity between the chorionic epithelial layer and several of the islands, and here the picture presented was strongly suggestive of epithelial downgrowth with pearl formation.

The cells of the epithelial islands were actively dividing and had the same general heterogeneity as those of the chorionic epithelial layer. This is well seen in Fig. 4, which shows a high power view of one such island.

The cells of the islands commonly adopted a concentric lamellar pattern, but no cornification was seen in the centre of the islands.

The mesoderm showed intense activity in most of the nodules. There was marked fibroblastic proliferation in the region of the base of the ulcer and, being embryonic, the mesenchymal cells showed an irregular pattern and many cellular irregularities. In some cases these irregularities gave the tissue a sarcomatous appearance (Fig. 5).

There was considerable oedema of the mesodermal tissue of the nodules and collections of lymphocytes and heterophile polymorphs were common, particularly in the region of the epithelial islands.

The allantoic epithelium lining the nodules showed even more hyperplasia than the chorionic epithelium. In many nodules, the hyperplasia developed to the point of formation of small papillae with arborescent fronds extending into the allantoic cavity (Fig. 6). The cells forming the tips of some of the fronds showed degererative changes which mimicked malignant changes.

In summary, the nodules consisted of ulcerative lesions in which three features were prominent, (*a*) chorionic and allantoic epithelial regeneration and hyperplasia, (*b*) formation of epithelial islands in the mesoderm and (*c*) excessive mesodermal proliferation.

Results from different types of inocula.

The nodules described above developed following the inoculation of emulsified normal chorioallantoic membranes. Passaging of membranes bearing such lesions again produced lesions of the same type. The results recorded in Table I were those obtained following ten such serial passages.

When sterile nutrient broth was used as inoculum, similar lesions were obtained, but were smaller and less frequent.

When physiological saline was used as inoculum, small nodular lesions were occasionally obtained.

Investigation of factors in development of lesions.

In view of the observation that nodules frequently occurred opposite nodules of paraffin on the inside of the shell, it was suspected that the paraffin might be a factor in their development.

Accordingly, eggs were inoculated, without the use of paraffin, and sealed with plastic tape. However, nodular lesions were still obtained, although their incidence was decreased. Paraffin, although encouraging nodular formation, was, therefore, considered to be unnecessary for their development.

The possibility was considered, early in the investigation, that the nodules might have resulted from the active proliferation of intact cells in the membrane emulsion following implantation on the recipient choriollantoic membrane. Later it was found that similar nodules could be obtained using simple nutrient broth.

Confirmatory evidence for the conclusion that intact cells were not necessary for nodule formation was obtained in the following way :

Batches of membrane emulsions were frozen to — 20° C. and — 70° C., heated to varying temperatures and subjected to filtration through Seitz filters (Size 9).

Table II records the results obtained.

As may be seen, none of these procedures eliminated the formation of nodular lesions.

TABLE II.—*Nodule Formation with Various Methods of Handling Emulsified Membrane.*

Method of handling emulsified membrane.	Number of eggs used.	Number of eggs showing lesions.	Eggs showing lesions. per cent.
Emulsified membrane untreated	26	10	38
Frozen. — 20° C.	53	20	38
Frozen. — 70° C.	30	5	17
Heated 80° C. 20 min.	18	2	11
Heated 100° C. 20 min.	9	3	33
Filtered (Seitz filter, size 9).	118	42	36

Elimination of penicillin and streptomycin from the inoculum did not affect lesion production.

It was concluded that the lesions resulted from a combination of trauma to the membrane during inoculation procedures, the subsequent regeneration on the part of the membrane being accentuated by heat stable and filtrable material present in normal chorioallantoic membranes. Paraffin played an accessory rôle in nodule formation.

DISCUSSION.

The chorioallantoic membrane of embryonated eggs has proved a useful tissue for the isolation and cultivation of many viruses. Its ready acceptance of foreign tissue grafts has provided a satisfactory means of cultivation of a variety of tissues, sometimes as a preliminary step in the isolation of viruses contained in the tissues concerned.

However, it must be appreciated that the use of this tissue has its pitfalls. It is part of an actively growing embryonic organism and, as such, its reactions to stimuli are of different type and intensity from those shown by adult tissues similarly handled.

The possibility must always be considered, therefore, that lesions obtained on the chorioallantois, following the inoculation of material under examination, are non-specific in type.

The nodular lesions described above are of particular interest when the virus being sought is believed to have carcinogenic potentialities. The nodules present a number of histological appearances suggestive of cancer tissue.

The epithelial hyperplasia with cellular irregularities, the formation of epithelial downgrowths and islands and the abnormal patterns of the mesodermal tissue must be clearly recognised as in no way indicative of specific changes, as has been claimed by some workers. (Ruyck, 1951, 1953; Campbell, 1949).

Because of the variety and frequency of non-specific lesions occurring on the chorioallantois, the appearance of lesions following inoculation of tissue extracts must be subjected to rigid control before being accepted as evidence for the presence of a virus.

Foremost amongst these controls is the parallel serial passage of normal chorioallantois at the same time as the serial passage of the suspected tissue is being

carried out. The ability to obtain consistent lesions on serial passage is often assumed to indicate the presence of a causative agent which is actively multiplying. It is, therefore, important to appreciate that this is not necessarily always the case, for the nodular lesions described above maintained a constant appearance on serial passage.

Two other criteria should also be applied. Firstly, a quantitative relationship should be demonstrable between the titre of the inoculum and the number of lesions obtained. Finally, heating of the inoculum should prevent lesion formation.

SUMMARY.

A nodular type of non-specific lesion of the chorioallantois of embryonated eggs has been described.

The nodules consist of hyperplastic chorionic and allantoic epithelial layers, intense mesodermal proliferation and epithelial islands in the mesoderm.

Nodule formation occurred following the inoculation of physiological saline, broth and emulsified normal chorioallantoic membrane.

Pre-treatment of the inoculated material by freezing, heating and filtration did not reduce the incidence of nodules.

The significance of the lesion is discussed.

I am indebted to Sir Macfarlane Burnet, F.R.S., for his helpful advice and criticism throughout this work and to Mr. Matthai of the University of Melbourne for the photomicrographs.

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PATHOLOGICAL CHANGES IN RATS AS A RESULT OF TREATMENT WITH MONOCROTALINE.

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IT has long been known that *Crotalaria* plants when present in pastures can cause poisoning among livestock, resulting in pathological changes, especially in the liver and in the lungs. The disease of sheep and horses known as jagziekte in South Africa, and characterised by tumour-like proliferation of the bronchioles, has been claimed to be reproduced by feeding *Crotalaria dura* Wood and Evans (Theiler, 1920) or *Cr. globifera* E. May, (Marais, 1944) to horses, and by drenching (feeding by stomach tube) sheep with suspensions of *Cr. dura* (Steyn and de Kock, 1932).

As part of a systematic study of alkaloids from plants used as herbal remedies in underdeveloped countries, the effects of monocrotaline in young rats have been investigated.

Monocrotaline is the alkaloid isolated from *Crotalaria spectabilis* Roth. and *Cr. retusa* Linn. It belongs to the group of pyrrolizidine (Senecio) alkaloids known to be hepatotoxic (Henry, 1949). Its structure has been elucidated by Roger Adams and his associates, and proved to be the monocrotalic acid ester of retronecine (Adams, Shafer and Brown, 1952) (Fig. 1). According to Chopra

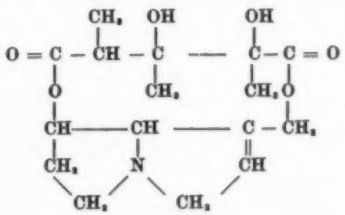

FIG. 1.—Monocrotaline.

(1933), in India, *Cr. retusa* Linn. is used for impetigo and scabies, and other *Crotalaria* species as laxatives, emmenagogues, in stomach troubles, etc. *Crotalaria* plants (of which about 600 species are known) are also used in Africa as herbal remedies in malaria, dysentery, black water fever, anthrax, dropsy, chronic cough, etc. (Watt and Breyer-Brandwijk, 1932).

EXPERIMENTAL.

Young Wistar rats bred in our animal house were used. In some experiments single litters were treated as a group, in long term experiments several contemporary litters were separated according to sex, and kept in metal cages 2 to 5 per

cage. The animals were fed on Shearer's Pig Weaner Nuts No. 1, as used in previous experiments of this series (Schoental, Head and Peacock, 1954) and water (or the appropriate solutions of the alkaloid) *ad libitum*. The weights of the animals were recorded at approximately weekly intervals till death. The progress of the pathological changes was followed by liver biopsy (by laparotomy under ether anaesthesia) or by killing animals at intervals, or when they were moribund. All the animals were examined *post mortem*, their organs were fixed in formal corrosive, and the sections stained with haematoxylin and eosin. Frozen sections fixed in 10 per cent formalin were stained with Sudan IV to reveal fat, and with acid ferrocyanide and carmalum to demonstrate haemosiderin. Blood haemoglobin was estimated by the method of Sahli; total serum proteins by the copper-sulphate specific gravity method. The apparatus of Flynn and de Mayo (1951) was used for electrophoresis of the serum on filter paper, and the recording Photodensitometer of Joyce, Loebl and Co. for the tracing of the serum protein patterns.

Pure crystalline monocrotaline, a generous gift from Dr. Roger Adams, University of Illinois, Urbana, U.S.A., was dissolved with the aid of equimolecular amounts of dilute hydrochloric acid in the appropriate volume of water, and the solutions stored at 0·4° C. till used. A saturated solution of monocrotaline in ethyl alcohol was used for skin applications. Fifty-three young rats, males and females, were used in the following series of experiments. Twenty others were kept as controls.

The experimental series comprised:

1. *Skin applications.*—A saturated solution of monocrotaline in alcohol was applied from a dropping pipette to the intrascapular region of 13 rats, 22–24 days old. The hair was clipped with scissors before application, care being taken not to damage the skin. The treatment was repeated 5 times weekly during 5 weeks.

2. *Injections.*—Fourteen rats, of which 7 were 2 weeks old and 7 were 4 weeks old, received single intraperitoneal injections of a 1 per cent watery solution of monocrotaline, in graded doses 0·5–4 mg. per rat.

3. *Feeding.*—(*a*) A female rat with a litter of six, was given solutions of monocrotaline containing 0·05 mg./ml., instead of drinking water, 3 days weekly during 3 weeks, beginning from the 8th day after the delivery.

(*b*) Six female rats 7 weeks old received solutions containing 0·03 mg./ml. of monocrotaline instead of drinking water 2 days weekly till death.

(*c*) Four male rats 7 weeks old were given solutions of monocrotaline containing 0·03 mg./ml., instead of drinking water, 2 days weekly during 1 year.

4. *Feeding and injections.*—Five female and 4 male rats 7 weeks old were given solutions of monocrotaline containing 0·03 mg./ml. instead of drinking water 2 days weekly during 5½ months. The animals were then injected intraperitoneally with monocrotaline (4 mg./rat) followed by a second injection (8 mg./rat) 3 weeks later.

RESULTS.

Regardless of the route of application, whether to the skin, by injection, or feeding, monocrotaline induced similar pathological changes in both sexes of young rats. The effects of treatment with monocrotaline depended mainly on the age of the animals and on the length of their survival. Immature animals

were particularly sensitive to the action of the alkaloid, which affected their growth and nutritional status.

1. *Skin applications.*

While no local skin changes were noticeable at the site of application of alcoholic solutions of monocrotaline, the alkaloid induced acute pathological changes in internal organs of the animals, mainly in the liver and in the lung, which led to death in the course of 4–6 weeks. Only 2 out of the 13 rats survived just over 3 months from the beginning of skin applications and about 8 weeks after the applications were discontinued. Similar changes were seen in all the animals which died early. The animals were emaciated and listless. Bleeding from the nostrils, rapid shallow breathing and cyanosis were present in the terminal stages. Their haemoglobin levels were above 80 per cent (Sahli). *Post mortem*, variable amounts of serous fluid were present in the pleural cavity, the lungs were enlarged, emphysematous and oedematous, of a spongy consistency, and did not collapse on pressure. Areas of haemorrhage, varying in size from small petechiae to patches of dark red or brown colour, in some animals involving whole lobes, or whole lungs, were present (Fig. 2). The thymus was usually small and the peribronchial lymph glands dark and enlarged. The heart showed no gross abnormality. In the abdomen only a little serous fluid was occasionally present. The livers were congested, slightly enlarged, firm, dark or mottled. All the blood vessels were congested ; the kidneys were dark, the spleens were slightly granular and sometimes enlarged. The pancreas was occasionally oedematous or white and firm. The small intestines were usually filled with mucus, which was sometimes stained with bile or blood. The Prussian blue macroscopic reaction for stainable iron was positive in the cortex of the kidneys, in the dark patches of the lungs, and sometimes also in the spleen and liver.

Microscopically, the lungs in all cases showed areas of haemorrhage into the alveoli. Some alveoli were packed with blood corpuscles and others contained eosinophil homogeneous material. The blood vessels were congested and dilated and in some, thrombosis was present (Fig. 3) with adjoining areas of infarction. Many macrophages contained haemosiderin. In one rat which died after six weeks in this series, and in a few in the feeding experiments, some epithelialisation of the lining of the alveoli (Fig. 4) and proliferation of the lining of the bronchioles (Fig. 5) was noted around areas of infarction.

In 3 male and 3 female rats the livers showed diffuse zonal haemorrhagic necrosis around the central veins (Fig. 6), surrounded in two cases by a zone of fatty degeneration and leaving only a narrow rim of hepatic cells in the periportal region. The areas of hyperaemia extended to the surface of the liver forming wedge-shaped zones which alternated with areas of rather hyperplastic parenchymal cells and giving a granular appearance to the surface of the liver (Fig. 7). In the majority of cases the portal and hepatic veins were dilated and congested and some were thrombosed (Fig. 6). Hyperplasia of hepatic cells round the portal systems was found in most cases.

Stainable iron pigment was seen in a few Kupffer cells and in large macrophages round the portal triads.

The kidneys were hyperaemic and in 4 of the 7 cases stained for iron, pigment granules were seen in the first convoluted tubules (Fig. 8).

The spleens were congested and in one iron pigment was seen in a few macrophages. Haemopoiesis was noted in most cases probably because the animals were very young.

The pancreas showed in some cases closely packed acini and faint staining of the secretory granules.

One of the two rats which survived 3 months from the beginning of skin applications died, showing extensive anasarca. It had no ascites or pleural effusion. The lungs showed a few dark pinhead spots, the peribronchial lymph nodes were enlarged and congested. The liver was dark, slightly swollen, and granular. The kidneys were very dark ; the spleen and the pancreas showed no gross abnormalities.

Microscopically, the lungs showed a few areas of haemorrhage in which crystals of haematoidin were present. There was some desquamation of the lining of the bronchi. The peribronchial lymph nodes showed red blood cells in the sinuses. The liver showed congestion and dilatation of the sinusoids and of the central veins, with some fatty change surrounding these areas, and there was slight hyperplasia of hepatic cells round the portal triads in which a little cellular infiltration was seen. Some thrombosed vessels were noted and in one section areas of haemorrhage round the central veins which extended to the surface in wedge-shaped form. The kidneys showed some lobulation of the glomeruli and some catarrh of the tubules, and much congestion of the blood vessels. The spleen was very congested, and megakaryocytes and pigmented macrophages were seen. In the pancreas the blood vessels were also congested. In the subcutaneous tissue much oedema was seen and areas of round-celled infiltration of the dermis.

The second rat killed the following day was still in good condition, had no oedema, but about 2 ml. of pleural effusion and a similar amount of ascites was present. The other pathological findings resembled those described above.

2. *Injections.*

Intraperitoneal injections of monocrotaline into immature rats in graded doses, 0·5–4 mg./rat in each age-group, resulted in their death in the course of 5 weeks after the injections. The 2 weeks old animals died mostly after 2–3 days, while the 4 weeks old rats survived 4–5 weeks, with the exception of 1 rat which survived only 3 days after the injection. The length of survival of individual rats in each of the two groups had little relation to the amount of monocrotaline injected. The animals became emaciated, bled from the nostrils, and showed pathological changes macro- and microscopically similar to those encountered in the rats which received the alkaloid by skin application. In the rats which died 2–3 days after the injections, centrilobular haemorrhagic necrosis and midzonal fatty changes in the liver were more pronounced than in those which died after several weeks.

3. *Feeding.*

Striking difference in the response to treatment with monocrotaline by feeding was noticed, depending on the age of the rats at which the treatment was begun.

(*a*) A female and its litter consisting of six 8 days old rats were given solutions of monocrotaline instead of drinking water 3 days weekly during 3 weeks. This treatment resulted in the death of all the animals in the course of two months from

the beginning of feeding. The young rats were smaller than the controls, listless, and had difficulty in breathing. The macro- and microscopical changes found *post mortem* in these animals were similar to those seen in animals which survived for a similar time after treatment by the other routes.

(*b*) and (*c*) However, when the feeding was started when the rats were 7 weeks old, all the animals survived for more than 7 months. They grew normally and seemed in good health. Liver biopsy taken from some animals after 6 weeks and after 13 weeks of feeding showed slight round-cell infiltration of the portal areas, dilatation of the sinusoids, congestion of veins, some of which were thrombosed, vacuolation of liver cells, and slight regeneration. The female rats died in the course of the following 4 months. They rapidly lost weight in the last weeks before death, became emaciated, cyanotic, dyspnoeic and some had epistaxis.

Post mortem all the subcutaneous and visceral blood vessels were congested. The lungs were congested with dark red patches here and there. Pleural effusions were present in some of the animals. One rat had bronchopneumonia. The livers were dark, congested, and slightly granular; the spleens and kidneys were dark and congested. In some animals haemolymph nodes were present. All these organs gave macroscopic Prussian blue reaction for iron.

Microscopically, the lungs showed petechial haemorrhages, where blood corpuscles and exudate of plasma filled the alveoli as in (Fig. 4). Some hyperplasia of the epithelium lining the alveoli and of the terminal bronchioles was seen at the margin of the haemorrhagic area in 3 rats, which survived 9–10 months of treatment. The last rat of this series which died after $10\frac{1}{2}$ months of treatment had also bronchopneumonia and bronchiectasis. The livers showed vascular hyperaemia most marked round the central veins. Thrombosis was noted in the large vessels. In one rat there was necrosis fairly evenly distributed throughout the liver. Sections stained for iron showed deposits of haemosiderin in the Kupffer cells and smaller granules in the hepatic cells. The kidneys showed marked hyperaemia and large granules of stainable iron were present in the cells lining the proximal convoluted tubules. In the spleens large amounts of haemosiderin were seen in macrophages in the red pulp (Fig. 9).

All the male rats in the chronic feeding experiments survived more than a year, grew normally and remained in apparent good health. One of these rats was killed one year after the beginning of feeding with monocrotaline. It was in very good condition. *Post mortem*, the lungs showed firm, grey patches, and the liver had a slightly granular and mottled surface. The lobe of the liver from which a biopsy specimen was taken 9 months previously showed still distinctly the cut straight edge.

Microscopically the lungs showed patches of chronic bronchopneumonia, and in the liver thrombosed vessels and hydropic vacuolated hepatic cells were present. The spleen was congested and many pigmented macrophages were seen in the red pulp.

4. *Feeding and injections.*

The female rats in this series died 7–9 months after the beginning of feeding and 1–3 months after the intraperitoneal injections of monocrotaline. The pathological changes found *post mortem* were similar to those seen in rats of Series 3*b*, which received only prolonged feeding with monocrotaline.

The male rats in this series remained in good health for more than a year.

Control rats.

No pathological changes of the type described, were seen in control rats, which were killed at ages corresponding to those of the experimental animals.

In some of the experimental and control animals the blood taken from the tail vein was examined for its haemoglobin content, fragility of the red corpuscles and in stained film. The fragility of the red cells and the haemoglobin content of the blood of experimental rats were in the same limits as those of the control animals (mean 0·4 per cent NaCl and 92 per cent Hb respectively). In some instances the blood film of the experimental rats showed the presence of nucleated red corpuscles and marked polychromasia.

Total serum proteins were determined in the blood taken from the carotid vessels. The values found in experimental animals were within the same limits as those found in control rats (mean 6·7 g./100 ml.) except for one animal which had the low value of 5 g./100 ml. Electrophoresis on paper disclosed decrease of the albumin and a relative increase of the globulin fractions in the sera of the experimental rats, as compared with those of the control animals.

DISCUSSION.

The results of these experiments indicate that monocrotaline, like isatidine (Schoental, Head and Peacock, 1954) induces pathological changes in internal organs of rats regardless of the route of application, whether to the intact skin, by

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EXPLANATION OF PLATES.

FIG. 2.—1158/54. Dark, congested and oedematous lungs (*a*) of a rat from Series 1 which died 4 weeks after the beginning of skin applications of monocrotaline. Compare with lungs (*b*) from a control rat of the same age and size. The white patches on the lungs are high lights.

FIG. 3.—1208/54. Section of lung of a rat from Series 1 which died 6 weeks after the beginning of treatment, showing thrombosed blood vessels and haemorrhage into the alveoli. H. and E. × 50.

FIG. 4.—1059/54. Section of lung of a rat from Series 2 killed 5 weeks after the injection, showing serous exudate into the alveoli, some of which show hyperplasia of lining cells. H. and E. × 73.

FIG. 5.—1208/54. Papillary-like structures of bronchial lining. The same section as Fig. 3. H. and E. × 60.

FIG. 6.—1208/54. Section of liver of the same rat showing central haemorrhagic necrosis, thrombosed vessels, and absence of proliferation of endothelial cells in veins. H. and E. × 25.

FIG. 7.—1128/54. Section of liver of a rat which was killed 1 month after skin applications of monocrotaline, showing areas of central haemorrhagic necrosis extending to the surface, and hyperplasia of the surviving cells. H. and E. × 55.

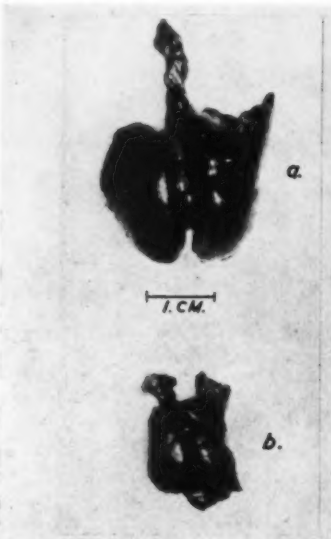
FIG. 8.—1143/54. Section of kidney of a rat from Series 1, which was killed after 1 month of skin applications, showing distribution of stainable iron pigment in the cells lining the proximal convoluted tubules. Ferrocyanide and carmalum. × 150.

FIG. 9.—968/54. Section of spleen from a rat which died after 9 months of feeding monocrotaline, Series 3*b*, showing the distribution of stainable iron pigment in macrophages in the red pulp. H. and E. × 42.

FIG. 10.—616/53. Section of lung from a female rat which died 23 months after the beginning of treatment by feeding with retrorsine, showing a papillary adenoma. H. and E. × 46.

FIG. 11.—687/51. Section of liver from rat treated with isatidine, showing endothelial proliferation of hepatic veins. Note the absence of such changes in the portal veins. H. and E. × 60.

FIG. 12.—43/52. Section of lung from a rat treated by feeding with isatidine, which died 6 weeks after the beginning of treatment, showing endothelial proliferation of capillary blood vessels. H. and E. × 60.



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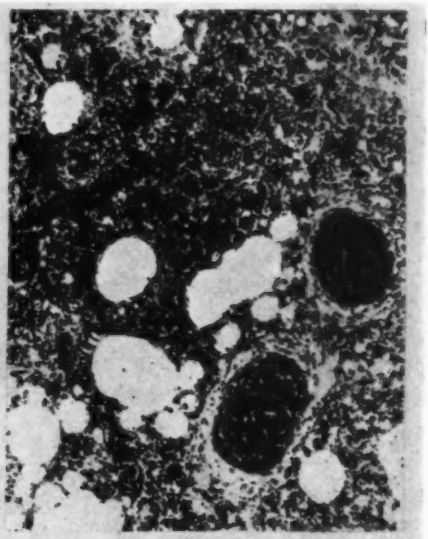

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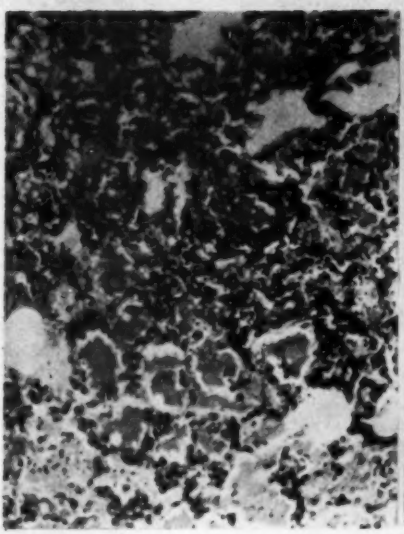

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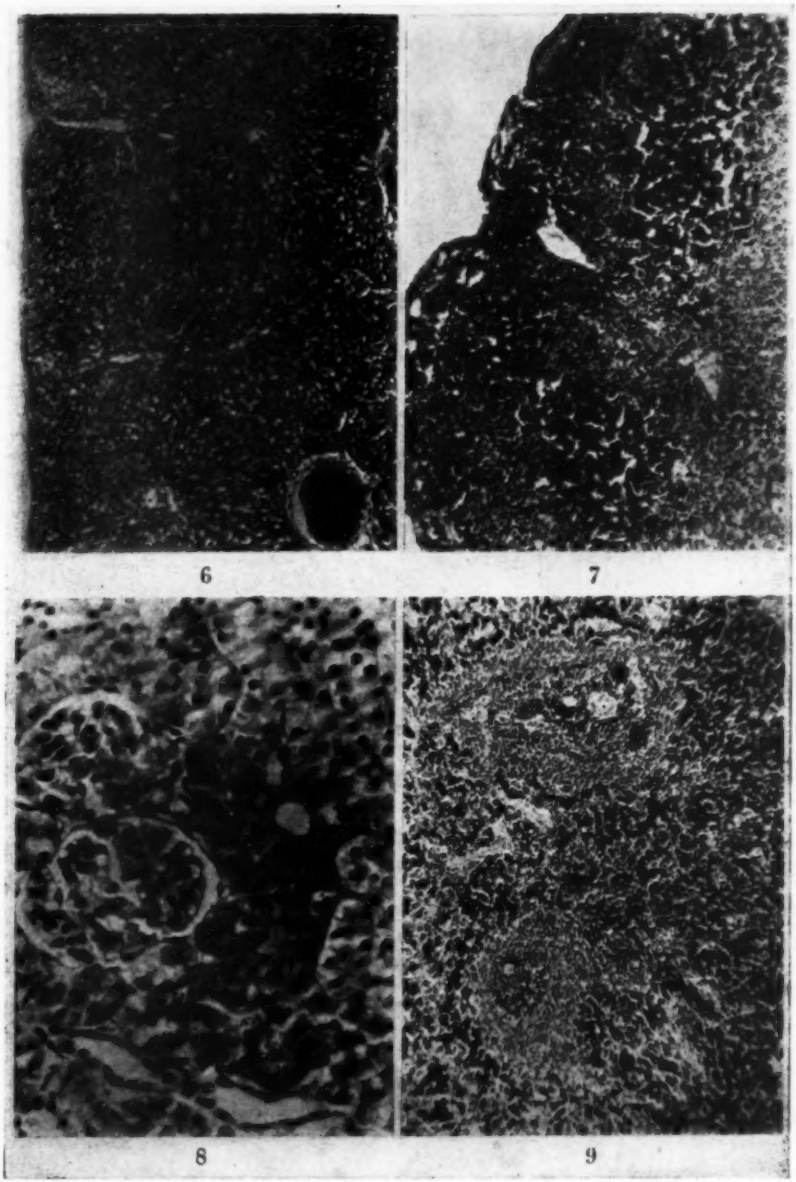

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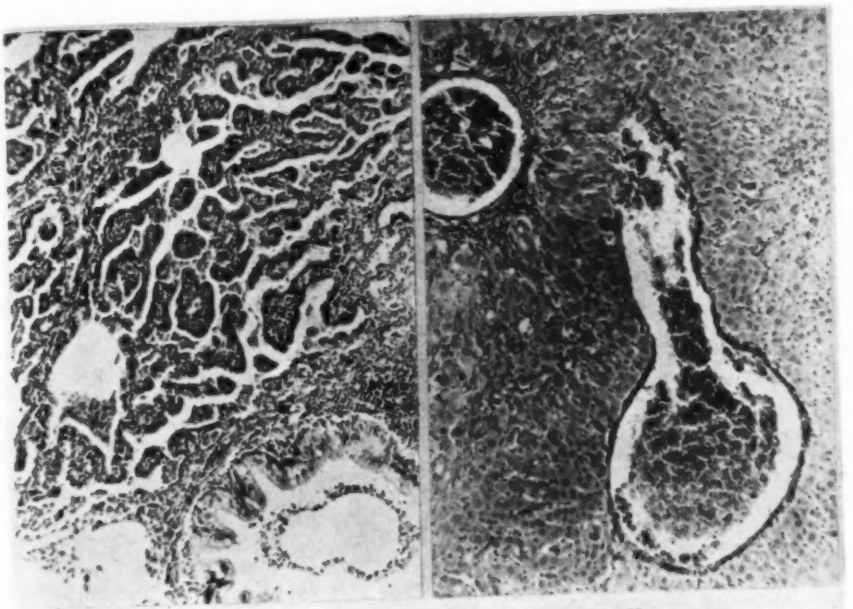

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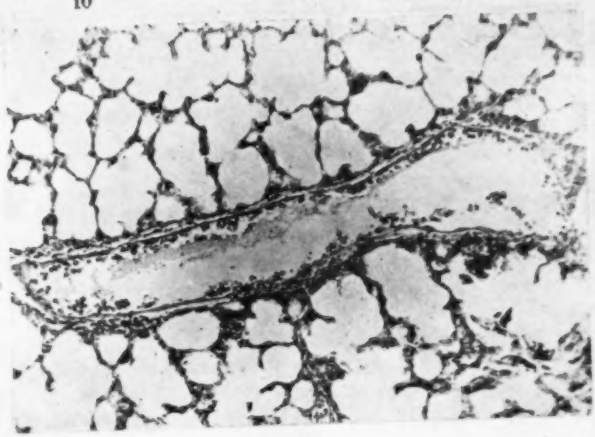

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Schoental and Head.

injection, or by feeding. However, due to the unpalatability of this alkaloid, only very dilute solutions containing 0·03 mg./ml. were consumed by adult rats, in which only chronic and mild changes developed.

Rose *et al.* (1945) using single subcutaneous injections determined the acute toxicity of monocrotaline for rats (L_d — 91·7 mg./kg.) and for mice (L_d — 261·3 mg./kg.) The pathological changes recorded by these workers included pulmonary oedema in the majority, and hydrothorax and ascites in some of the animals. Central haemorrhagic necrosis, congestion of the sinusoids, and hyperplasia of cell cords were seen in the livers, and there was no leucocytic reaction.

Similarly, Ratnoff and Mirick (1949) reported congestion and oedema of the lungs, central necrosis of the liver, and some necrotic changes of the renal tubules as the main pathological changes in rats injected subcutaneously with monocrotaline.

While these workers described the pathological changes in mice and rats resulting from subcutaneous injections and from feeding of monocrotaline, no records are available of the effects following applications of this alkaloid to the skin of experimental animals. In view of the recorded uses of *Crotalaria retusa* Roth. externally for skin disorders (Chopra, 1933), it seemed important to test whether monocrotaline could induce pathological changes when absorbed through the skin. The results, in rats, here reported show clearly that this is the case. Thus, the potential danger involved in the application of extracts of *Crotalaria* plants to the skin is similar to that which follows their consumption.

In the present experiments two types of pathological changes could be distinguished, acute or subacute ones, in immature animals which survived up to about 6 weeks after the beginning of treatment by all three routes (skin applications, injections, and feeding); and chronic changes induced by prolonged intermittent feeding of monocrotaline to rats, started when the latter were 7 weeks old.

In the published studies of the action of monocrotaline adult animals have been used; while we investigated mainly the effects resulting from treatment with this alkaloid of immature rats, some of pre-weaning age. These were very sensitive to the action of the alkaloid, and developed acute changes, consisting of congestion and oedema of the lungs, which contained almost invariably various degrees of haemorrhage in the alveoli, some thrombosed vessels, and often infarcts. These degenerative changes were accompanied in some animals which survived about 6 weeks or longer, by proliferation of the bronchial lining and by epithelialisation of the alveoli. This was probably an attempt at regeneration after the damage due to the earlier haemorrhage and oedema. Montgomery (1944) described similar regenerative features in the early stages of repair after mechanical injury, which he produced by cutting out, aseptically, wedge-shaped pieces of lung in cats. Probably any lung injury, whether caused by a mechanical, bacterial or chemical agent, stimulates an active proliferation of surrounding epithelial and connective tissues. So far, we have no evidence whether these early proliferative changes in the lungs of rats treated with monocrotaline may give rise to neoplasia. In previous experiments in which rats were treated with retrorsine for more than a year, one of the female animals which did not show pronounced liver changes developed a primary lung tumour (Fig. 10) (Schoental, Head, and Peacock, 1954); its significance however cannot be evaluated at present.

Congestion of all blood vessels and thrombosis in some of them was a general and constant feature in all animals, and the pathological changes in the lungs and other organs were probably the result of it. Thus the livers were congested and showed central haemorrhagic necrosis and some midzonal fatty infiltration, dilatation and congestion of the sinusoids, and thrombosed vessels ; the liver cells showed hydropic vacuolisation and some regeneration.

Rose *et al.* (1945) determined the prothrombin time after injections into rats of monocrotaline and of other *Senecio* alkaloids. They found that the normal value of 39·8 seconds was prolonged up to 1800 seconds in rats 24 hours after the injections of monocrotaline (120 mg./kg.).

It is of interest that in spite of the emaciation of the animals and of the haemorrhages, the values of haemoglobin (mean 92 per cent by the Sahli method) and those of total serum proteins (6·7 g./100 ml.) were rather high but still in normal limits as compared with control rats. In rats treated with isatidine various degrees of anaemia and low values of total serum proteins have been found (Schoental, 1954).

The effects of treatment of young rats with monocrotaline, when compared with those due to isatidine, and a mixture of *S. Jacobaea* alkaloids show some similarities and some marked differences. The former included the acute necrotic changes in the liver. After the treatment with monocrotaline congestion of blood vessels and the presence of thrombi in the lung and liver, and lung oedema were more prominent. On the other hand, proliferation of bile ducts, cellular infiltration round the portal systems, and endothelial proliferation of the walls of hepatic veins (Fig. 11) and of blood vessels in the lungs (Fig. 12) were a common feature in animals treated with retrorsine, isatidine, and the mixture of alkaloids from *S. Jacobaea*, Linn (Schoental, Head, and Peacock, 1954).

Till some information is available on the subject of the mechanism of action of the various *Senecio* alkaloids, attempts to explain such differences in response to them could at present be only speculative. In long term feeding experiments a prominent feature of the chronic changes was the presence of stainable iron in all the organs examined : lungs, lymph nodes, liver, spleen, kidneys. Occasionally crystals of haematoidin were present in the lungs.

In the animals in which liver biopsy was taken 6 and 13 weeks after the beginning of treatment, the livers showed *post mortem* easily recognisable sites from which the specimens were cut. The sites of liver biopsy taken at similar times from control rats were rounded and regenerated.

SUMMARY.

Pathological changes in rats resulting from treatment with monocrotaline by skin application, by injections, and by feeding, are described, and compared with those seen in rats after treatment with other *Senecio* alkaloids.

The potential danger involved in the use, in some countries, of *Crotolaria* plants as herbal remedies, whether by ingestion, or externally by skin application, is pointed out.

ADDENDUM

The 6 male rats in series 3C and 4 which remained healthy for over a year were then injected intraperitoneally with 12 mg. of monocrotaline. Five died and 1 was killed from 2½ to 3 months later. Two had developed liver tumours similar

to those produced by the *Senecio* alkaloids described by Schoental, Head and Peacock (1954). One of these had a single tumour 5 mm. diameter of endothelial origin and the other had multiple trabecular hepatomata 2-3 mm. diameter; in this rat their was some fibrosis and much bile duct and endothelial cell proliferation. A papillary adenoma was present in the lung of a third rat.

This work has been supported by a grant from the British Empire Cancer Campaign. We are greatly indebted to Dr. Roger Adams, University of Illinois, Urbana, for a generous gift of monocrotaline, and to Dr. P. R. Peacock for his interest and criticism. Our thanks are due to Mr. S. Breslin for the photographs, to the Pathology Technical Staff for the histological sections, to Mr. C. Bern also for his part in microphotography, to Mrs. J. Jack for the protein estimations, to Mrs. J. Rae for valuable technical assistance and care of the animals, and to the Staff of the Animal House for help.

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SURVEY OF PAPERS

McKenzie discusses the effect on cancer mortality rates of an increase in the duration of survival from cancer at certain typical sites. He finds that the resulting changes in the rates are not constant and vary with the form of the mortality curve (p. 1).

Rennæs and Østberg report an investigation into the relationship of stomach cancer mortality to social conditions in Oslo (p. 7).

Jelliffe and Thomson have studied a series of 227 cases of Hodgkin's disease and discuss the factors which influence the prognosis. These include the stage of the disease at the time of presentation, the age of onset, the sex of the patient and the histological appearance of the lesion (p. 21).

Thomson postulates that Hodgkin's disease is a tumour arising in and metastasising from the thymus gland (p. 37).

Chesterman has injected human tumour tissue intracranially into mice and obtained infiltrative growth in a small number of animals (p. 51).

Boyland, Wallace and Williams find that patients with cancer of the bladder have higher urinary β-glucuronidase and sulphatase concentrations than are found in subjects free of malignant disease. This finding supports the hypothesis that the action of these urinary enzymes is a contributary cause of bladder cancer, liberating carcinogenic aminophenols or other compounds from inactive conjugates (p. 62).

Bielschowsky reviews our knowledge of the effect of variations in hormone levels on neoplasia (p. 80).

Doniach and Logothetopoulos have investigated the thyroid and its function in rats three months after treatment with radioactive iodine. They find a reduction in the weight of the gland, but maintenance of the normal body weight and thyroid ability to take up iodine, increased follicle cell height and ability to hypertrophy. The response to a goitrogen such as propylthiouracil is diminished, with a reduction in mitosis and formation of bizarre nuclei. They find the diminished goitrogenic response to be a sensitive test for radiation damage (p. 117).

Marchant and Orr describe and discuss a series of experiments on mice involving transference of skin grafts at various stages in the process of carcinogenesis by methylcholanthrene and croton oil treatment (p. 128).

Clemo, Miller and Pybus describe the production of skin tumours in mice by the local application of chemical fractions separated from industrial smoke (p. 137).

Miller and Pybus have crossed reciprocally two inbred strains of mice which differ in their incidence of spontaneous papillomata of the forestomach. They find that the incidence of these tumours in the hybrids does not vary with the direction of the cross and is the same as that of the more susceptible parent strain, but that the tumours appear much later, as in the other parent strain. Some of the mice given a subcutaneous injection of methylcholanthrene showed an increased incidence of more numerous, more malignant and earlier appearing stomach tumours (p. 142).

CAMPBELL reports the occurrence of multiple primary epithelial tumours in 3 out of 5 fowls given 2-acetamidofluorene by mouth four years previously (p. 163).

WALPOLE, WILLIAMS and ROBERTS find a high incidence of bladder tumours in rats given 3 : 2′-dimethyl-4-aminodiphenyl by subcutaneous injection. The difference in incidence in the two strains of rats used is discussed (p. 170).

ROE and SALAMAN record the results of screening a series of substances for powers of initiating carcinogenesis, such as is possessed by urethane. The majority of the substances tested on mice have one or more biological activities in common with urethane, or are related to it chemically (p. 177).

PEARSON and POWELL find that acenaphthequinone-bisulphite significantly inhibits the growth of transplanted tumours in mice. When irradiation treatment is given concurrently the inhibitory effect is greater than the simple summation of the effects of the two agents acting independently (p. 204).

AMOS, GORER and MIKULSKA present genetic data suggesting that the increasing virulence shown by transplanted tumours may be due to antigenic simplification (p. 209).

AMOS finds that when antibodies against a C57 Black leukosis E.L.4 are inoculated into non-immune homologous mice they can be detected in the bloodstream for as long as 12 days (p. 216).

METCALF describes a nodular type of non-specific lesion of the chorioallantois of embryonated eggs. The nodules consist of hyperplastic chorionic and allantoic epithelial layers, intense mesodermal proliferation and epithelial islands in the mesoderm. The significance of the lesions, the incidence of which is not reduced by freezing, heating or filtration of the inoculum, is discussed (p. 222).

SCHOENTAL and HEAD describe the pathological changes found in rats treated with monocrotaline, and compare them with those produced by other *Senecio* alkaloids (p. 229).